Safety Assessment of Copper Gluconate as Used in Cosmetics

Status:	Draft Tentative Report for Panel Review
Release Date:	September 6, 2024
Panel Meeting Date:	September 30 – October 1, 2024

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume, M.B.A. This safety assessment was prepared by Preethi Raj, M.Sc., Senior Scientific Analyst/Writer, CIR.

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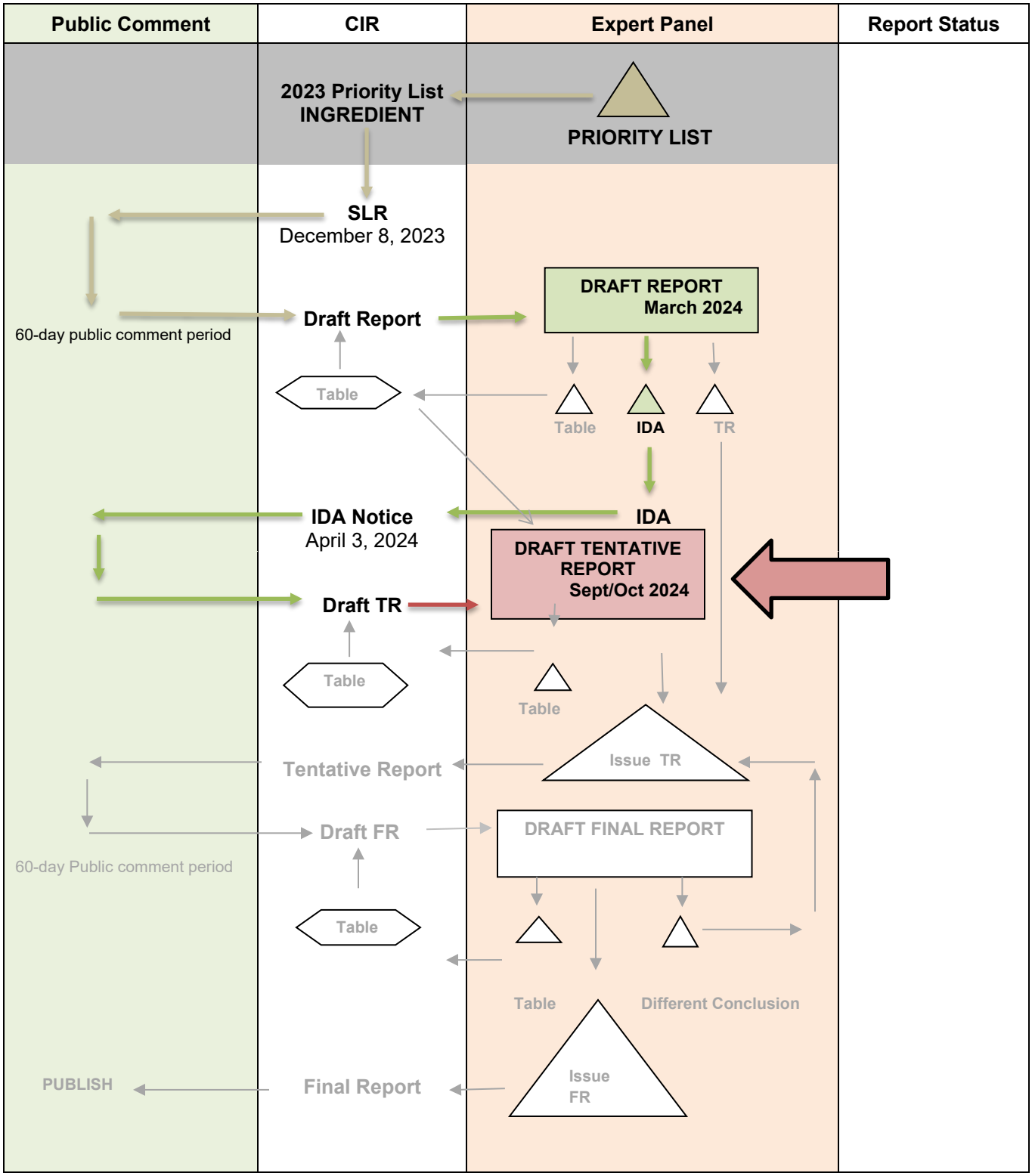
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Copper Gluconate

MEETING September/October 2024



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Preethi S. Raj, M.Sc.
Senior Scientific Analyst/Writer, CIR
Date: September 6, 2024
Subject: Safety Assessment of Copper Gluconate as Used in Cosmetics

Enclosed is the Draft Tentative Report of the Safety Assessment of Copper Gluconate as Used in Cosmetics (identified as *report_CopperGluconate_092024* in the pdf). This is the second time the Panel is seeing a safety assessment of this ingredient. At the March 2024 meeting, the Panel issued an Insufficient Data Announcement (IDA). The Panel identified the following data needs to determine the safety of this ingredient:

- Impurities data for Copper Gluconate as used in cosmetics
- Dermal irritation and sensitization data at maximum concentration of use
- Ocular irritation data, if available

The following data were received in response to the IDA and have been incorporated into the report (highlighted in report)
data1_CopperGluconate_092024

- Personal Care Products Council. 2024. Updated Concentration of Use by FDA Product Category: Copper Gluconate

data2_CopperGluconate_092024

- Anonymous. 2021. Product sheet (Copper Gluconate, USP powder)
- Anonymous. 2024. Certificate of analysis (Copper Gluconate, USP powder)
- Anonymous. 2024. Elementary impurity profile (Copper Gluconate, USP powder)

data3_CopperGluconate_092024

- Anonymous. 2011. Clinical safety evaluation: repeated insult patch test (powder containing 0.1% Copper Gluconate)

data4_CopperGluconate_092024

- Anonymous. 2024. Regulatory statements – Copper Gluconate (elements)

data5_CopperGluconate_092024 (dose/area in parentheses)

- Anonymous. 2012. HRIPT of a leave-on baby product containing 0.00008% Copper Gluconate (0.04 μg/cm^2)
- Anonymous. 2013. HRIPT of a rinse-off baby product containing 0.2% Copper Gluconate (100 μg/cm^2)
- Anonymous. 2010. HRIPT of a rinse-off adult product containing 0.00008% Copper Gluconate (0.04 μg/cm^2)

data6_CopperGluconate_092024

- Anonymous. 2022. Topical application ocular irritation screening assay using the EpiOcular™ human cell construct (face cream containing 0.0025% Copper Gluconate)

The following documents are also included in the package for your review:

- a flow chart (*flow_CopperGluconate_092024*)
- ingredient history (*history_CopperGluconate_092024*)
- search strategy (*search_CopperGluconate_092024*)
- data profile (*dataprofile_CopperGluconate_092024*)
- transcripts from the previous meeting (*transcripts_CopperGluconate_092024*)

After reviewing these documents, the Panel should issue a Tentative Report with a safe, safe with qualifications, insufficient, unsafe, or split conclusion, and Discussion items should be identified.

CIR History of:

Copper Gluconate

July 2022

-Concentration of use data submitted by Council

January 2023

-FDA frequency of use data obtained

December 2023

-Copper Gluconate SLR posted on the CIR website

No new data were received.

March 2024

A Draft Report was being presented to the Panel for review. The Panel issued an IDA for the following data needs:

- Impurities data for Copper Gluconate as used in cosmetics •
- Dermal irritation and sensitization data at maximum concentration of use
- Ocular irritation data, if available

April, May, and July 2024

The following data were received in response to the IDA:

- Updated concentration of use data (submitted by Council)
- Copper Gluconate purity information, and regulatory statements for impurities
- 4 HRIPTs testing products containing up to 0.2% Copper Gluconate
- An in vitro ocular irritation test of a face cream containing 0.0025% Copper Gluconate

September/October 2024

A Draft Tentative Report is being presented for Panel review.

Copper Gluconate Data Profile* – September 30 – October 1, 2024 – Writer, Preethi Raj																													
				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Copper Gluconate	X	X	X	X		X	X	X			X			X	X			X				X			X				

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

[Copper Gluconate]

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Copper Gluconate	527-09-3	✓	✓	✓	NR	NR	✓	NR	NR	✓	NR	NR		✓*	NR		

Search Strategy – updated 08/13/2024

((((((((((((Copper Gluconate) OR (Bis(D-Gluconato)Copper)) OR (Copper, Bis(D-Gluconato)-)) OR (Cupric Gluconate)) OR (Gluconic Acid, Copper Salt)) OR (Actibronze)) OR (Cutein)) OR (Glycosnail VEG)) OR (Sepitonic M3)) OR (Sepitonic M4)) OR (Givobio GCu)) OR (Gluconal CU)) OR (OriStar CGC)) OR (527-09-3) – 88,516 hits/4 useful

LINKS

Search Engines

- Pubmed - http://www.ncbi.nlm.nih.gov/pubmed
  - appropriate qualifiers are used as necessary
  - search results are reviewed to identify relevant documents
- Connected Papers - https://www.connectedpapers.com/

Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/winci/ingredient-custom-search/
- FDA Cosmetics page - https://www.fda.gov/cosmetics
- eCFR (Code of Federal Regulations) - https://www.ecfr.gov/
- FDA search databases: https://www.fda.gov/industry/fda-basics-industry/search-databases
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras
- SCOGS database: https://www.fda.gov/food/generally-recognized-safe-gras/gras-substances-scogs-database
- Inventory of Food Contact Substances Listed in 21 CFR: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=IndirectAdditives
- Drug Approvals and Database: https://www.fda.gov/drugs/development-approval-process-drugs/drug-approvals-and-databases
- FDA Orange Book: https://www.fda.gov/drugs/drug-approvals-and-databases/approved-drug-products-therapeutic-equivalence-evaluations-orange-book
- OTC Monographs - https://dps.fda.gov/omuf
- Inactive Ingredients Approved For Drugs: https://www.accessdata.fda.gov/scripts/cder/iig/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- EUR-Lex - https://eur-lex.europa.eu/homepage.html
- Scientific Committees (SCCS, etc) opinions: https://health.ec.europa.eu/scientific-committees_en https://health.ec.europa.eu/scientific-committees/scientific-committee-consumer-safety-sccs_en
- ECHA (European Chemicals Agency – REACH dossiers) – https://echa.europa.eu/
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- EFSA (European Food Safety Authority) - https://www.efsa.europa.eu/en
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) IRIS library - https://apps.who.int/iris/
- a general Google and Google Scholar search should be performed for additional background information, to identify references that are available, and for other general information - www.google.com https://scholar.google.com/

Botanical Websites, if applicable

- Dr. Duke's - https://phytochem.nal.usda.gov/
- 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
- American Herbal Products Association Botanical Safety Handbook (2nd Edition; 2013) - http://abc.herbalgram.org/site/DocServer/AHPABotanicalSafety_FMexcerpt.pdf?docID=4601
- National Agricultural Library NAL Catalog (AGRICOLA) https://agricola.nal.usda.gov/
- The Seasoning and Spice Association List of Culinary Herbs and Spices
- http://www.seasoningandspice.org.uk/ssa/background_culinary-herbs-spices.aspx

Fragrance Websites, if applicable

- IFRA (International Fragrance Association) – https://ifrafragrance.org/
- Research Institute for Fragrance Materials (RIFM) - https://www.rifm.org/#gsc.tab=0 http://fragrancematerialsafetyresource.elsevier.com/
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MARCH 2024 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Belsito Team – March 28, 2024

DR. BELSITO: Here we have a Wave 2 comments from PCPC. I won't go through all the comments, but I agreed with them all. Did anyone have any disagreement with the Council's comments?

DR. SNYDER: I did not.

DR. KLAASSEN: No.

DR. RETTIE: No, they're fine.

DR. BELSITO: Right. Basically, this is the first time we're looking at this ingredient. An SLR was announced in December of 2023. We previously looked at gluconic acid, potassium gluconate, and sodium gluconate. In 2019 the final report was published with a conclusion that these ingredients are safe in the present practice of use and concentration in cosmetics, but we have never looked at copper.

In terms of impurities, we have -- well, looking at the document, the first impurities we have -- the purity of food-grade copper is said to be 98 to 102 percent. Limits for reducing substances is one percent. No other impurities data, so my question is, as we go forward, are we assuming that copper in cosmetics is food-grade in terms of the impurities? If so, we should state that in the discussion, or do we want impurities data for cosmetic-grade copper gluconate? Paul? Curt? Allan?

DR. SNYDER: Well, I took that impurities section, Don, just for them to say that was the only impurities data there was. I mean, not necessarily the -- so, I mean, that's the only impurities data we have.

DR. BELSITO: I know, but is it sufficient? I mean, we could just keep this angle and say it's insufficient for impurities data and give it to those guys. Are we going to do that, or are we going to assume that that's the data for cosmetic-grade?

DR. SNYDER: Well, again, we're talking about, for leave-ons, a 0.006 percent in eyeliners. It's an essential element. I mean, I didn't -- it didn't raise any red flags to me. I'll say that.

DR. BELSITO: Curt? Allan?

DR. RETTIE: Yeah, I was the same --

DR. BELSITO: I mean, what difference (audio skip) fault, not having impurities data on the cosmetic-grade or the impurities on food-grade?

DR. SNYDER: Impurities data in any context. It's only 0.006 percent. I mean, I couldn't even guess what a impurity might be in there that would be of concern. I mean, copper is a liver and kidney toxin at high levels, but I don't -- yeah. I guess I'd put it onto Curt. I mean, he has more experience in these types of questions than me.

DR. KLAASSEN: Yeah, again, the concentrations are quite low. It probably isn't absorbed well. There is one concern, theoretical concern, with copper. There are people that are genetically susceptible to copper toxicity called Wilson's disease.

DR. BELSITO: Mm-hmm.

DR. KLAASSEN: Yeah, I'm not overly concerned about that. It might be nice to at least mention that in the text, that there is -- some people are genetically susceptible. We do get copper exposure in our drinking water as water is going through copper pipes today. Copper isn't, well, a real -- as far as metals are concerned, copper is relatively non-toxic. It's not like a mercury or a lead or a chromium or a cadmium.

DR. BELSITO: I understand, but it's a mined mineral. Are we not concerned about, perhaps, a toxic substance that is 50 percent of cosmetic-grade copper? We don't have data on cosmetic-grade.

DR. KLAASSEN: Well, I think we should ask for it. Can we ask for it now?

DR. BELSITO: Yeah. I mean, this is the first time we're seeing it.

DR. KLAASSEN: Okay, that's what I thought. Let's ask for it.

DR. BELSITO: Okay.

DR. KLAASSEN: Also, what it's like -- in the cosmetic grade, what are the impurities?

DR. BELSITO: Okay. I'm fine with that.

DR. RETTIE: I mean, is it established that there's a cosmetic-grade separate from the regular grade?

DR. BELSITO: I don't know.

MS. FIUME: No, there's --

DR. KLAASSEN: We don't --

DR. BELSITO: They specifically said food-grade.

MS. FIUME: The term cosmetic-grade in the U.S. does not exist. Nothing is cosmetic grade.

DR. BELSITO: Right. We're ask --

MS. FIUME: It may be used in cosmetics, but.

DR. BELSITO: We're asking for the impurities in copper gluconate as used in cosmetics.

DR. RETTIE: Is the likelihood that what comes back from that is the impurities that we have already in the document here?

DR. BELSITO: We really have no impurities other than it says that the limit for reducing agents is one percent. I don't even know what the reducing agent is.

DR. RETTIE: Well, they put limits on lead in the food-grade on reducing substances.

DR. BELSITO: I couldn't hear what you said, Allan.

DR. RETTIE: Well, I'm just reading the impurities section, and there are limits for the food-grade copper of no more than five mgs per kgs in lead in a gram, and, similarly, some information on the limit for reducing substances of one percent.

DR. BELSITO: Right. What is the reducing substance?

DR. RETTIE: That's not specified.

DR. BELSITO: Yeah, I mean, this is the first time we're seeing it.

DR. RETTIE: Yeah, sure. Absolutely. We can ask for more.

DR. BELSITO: I (audio skip) what the impurities are there.

DR. RETTIE: Yeah.

DR. BELSITO: Paul is right. The leave-on is 0.006 percent in the eyeliner, 0.2 percent in the baby shampoo for rinse-off.

Then, under Exposure Assessment, they have 0.0025 percent in a body lotion, 0.1 percent in a makeup remover. That's because of, I'm assuming, we're now doing the systemic exposure based upon not only concentration, but body surface area to what it's applied. It's not entirely clear, even though it says, "product category with a higher level of exposure." I think it needs to -- I mean, it assumes we've read the exposure report and know where we're going with these margins of safety. This is PDF page 20. I think it should sort of say that highest use concentration but product category with a higher level of exposure based upon the concentration and the body surface area to which the product is exposed. It makes it a little clearer. You see what I'm talking about, Monice? It's the second par --

MS. FIUME: I'm sorry. The second paragraph?

DR. BELSITO: Yeah, second paragraph in the exposure assessment, PDF page 20.

MS. FIUME: Yes. Okay.

DR. BELSITO: Just make it clearer that because the highest use concentration, then it says, "or for the product category with the higher level of exposure," I think higher level of exposure based upon concentration of use and extent to which the product is applied to the body surface, or something like that.

DR. KLAASSEN: How is it the retention factor here lies at 0.01? Is that really based on data?

DR. BELSITO: There was --

MS. FIUME: It looks as if Jinqiu Zhu's not online.

DR. BELSITO: I was not part of the QRA subgroup that did that paper in 2008, Curt, so I can't answer how they came up with those retention factors and product categories. I don't even know if it was totally spelled out in the 2008 QRA paper how they came up with those retention factors.

I think I have a copy of it here. Let me see if I can pull it up. Okay. Oh, here it is. Retention factor. Yeah. Yeah. It never said how they came up with the retention factors. They just applied these retention factors.

I mean, I can tell you what they are by product category. Deodorants and antiperspirants are one. Shaving creams are one. Lip products are four. Eye products are two. Again, based upon increased absorption, body creams and lotions are 0.5. Facial creams for men are two because it's shaved skin. Toothpaste is oral. Rinse-off exposure is two. Mouthwashes are three. Nail care is 0.43. I don't know what the basis for those retention factors are. I'm sorry, I was reading the wrong numbers.

DR. RETTIE: Is it right? Is there any values as low as 0.01?

DR. BELSITO: Yeah.

DR. RETTIE: I'm just wondering if that's (audio skip) relative to 0.1 for other --

DR. BELSITO: 0.01 are -- Allan, let me go through -- are shave creams, depilatories, mouthwashes, shampoos, conditioners, bar soaps, liquid soaps -- they're all wash offs -- face washes, bath gels. So, 0.01 are rinse-off products essentially.

DR. RETTIE: Okay. Yep.

DR. BELSITO: 0.1 are mucosal products and sprays, so mucosa and inhalation.

DR. SNYDER: Monice has a comment, Don.

DR. BELSITO: Go ahead.

MS. FIUME: Don, reading the little paragraph above the values, I believe these may have actually come from Notes of Guidance. I don't see Jinqiu on our call right now. The retention values, I'm not sure if they're exactly the same between RIFM and the Notes of Guidance. I haven't compared it. I think, based on what he has there, he is using the Notes, that retention factors, so they may be similar, they may not. There might be a few discrepancies.

All it says about the retention factor was that it was introduced by the SCC-NFP to take into account rinsing off and dilution of finished products by application on wet skin or hair.

DR. BELSITO: Mm-hmm.

MS. FIUME: Again, it doesn't really, based on my quick look in here, describe how they've come up. As you say, it's rinse-off versus what it's being exposed to. Allan, if you're seeing a discrepancy, if you go back and look at the paper Don's looking at, if you see a discrepancy, that might be why. I'm not sure of the Notes of Guidance. It sounds like they're similar, but there may be a couple that are different.

DR. BELSITO: Yeah.

DR. KLAASSEN: Could those two pa- --

DR. RETTIE: No. I think -- I'm sorry.

DR. KLAASSEN: Are these two different papers that you're talking about? Don has one, and you have another?

MS. FIUME: I have the Notes of Guidance that were issued by the SCCS last year.

DR. KLAASSEN: Okay.

MS. FIUME: Don, is the paper you're referring actually the Api paper? Because the reference they use in Notes of Guidance is Hall (phonetic) et. al.?

DR. BELSITO: No. Mine is the Api paper.

MS. FIUME: It is the Api paper?

DR. BELSITO: I'm going to email it to you now.

DR. KLAASSEN: Yeah, I'd like to see that.

DR. RETTIE: Yeah.

DR. BELSITO: Paul Snyder, why am I not getting your email coming up?

DR. SNYDER: Just klpath@comcast.net -- klpath@comcast.net.

DR. BELSITO: Klpath -- oh, yeah. Okay. Curt --

DR. KLAASSEN: Hey.

DR. BELSITO: -- do you want curtklaassenphd@gmail?

DR. KLAASSEN: Yes. Yes.

DR. BELSITO: Okay. I just sent it.

DR. KLAASSEN: Thank you.

DR. BELSITO: It's page 15 of the paper is where they have the retention factors.

DR. KLAASSEN: Okay.

DR. BELSITO: Basically, I mean, when you look at them, leave-ons get a retention factor of one. Rinse-offs get a retention factor of 0.01 when they're mucosal or potentially inhaled -- well, 0.01, I'm sorry -- 0.1, right, when they're mucosal or potentially inhaled, and 0.01 for rinse-offs that are like shampoos, bath gels, et cetera. Those are pretty much the values.

DR. KLAASSEN: In essence, it's a hundred percent, 10 percent, and one percent.

DR. BELSITO: Right. Then I don't know if -- what was the reference that is in our report? Monice, let me see if it's in the RIFM report. It was Hall, you said?

MS. FIUME: Well, that's in the Notes of Guidance from SCCS. It's Hall et. al., 2007 and 2011.

DR. BELSITO: Okay.

MS. FIUME: Then, also, Stiling et. al., 2012, S-T-I-L-I-N-G.

DR. BELSITO: Yeah, none of those work. Yeah, Squire and Hall, 1985. No. No. They didn't reference them. They referenced a Squire and Hall, 1985 paper for determining the safety assessment factors. Okay. I honestly don't know how they differ.

MS. FIUME: They appear very similar. The Hall papers state, "European consumer exposure to cosmetic products," in both of their titles. It seems to be trying to make it specific to Europe. It seems as if the retention factors are similar. I think the only one I see different is there's one for toothpaste in the Notes of Guidance, and that's 0.05. All the others are 0.01, 0.1, and one.

DR. BELSITO: Yeah. Toothpaste would be a little higher than what RIFM would have at 0.01.

MS. FIUME: Yep.

DR. BELSITO: Okay.

MS. FIUME: Then mouthwash was 0.1.

DR. BELSITO: Okay. Is everyone clear on these retention factors? I mean, be that as it may, we have to know if it's been applied if we're doing absorption based upon --

DR. KLAASSEN: I mean, I think those numbers are realistic. I think they're useful. I'm not sure they're a hundred percent scientific, but they're good enough for risk assessment.

DR. BELSITO: Okay.

DR. KLAASSEN: I mean, we've always said -- we've always made a big deal if this is a wash-off -- the chemical's a wash-off or not.

DR. BELSITO: Mm-hmm.

DR. KLAASSEN: This is kind of giving the quantitative number to that.

DR. BELSITO: Uh-huh. The only issue I have was the exposure in a baby shampoo where they used the adult surface area for the head. We know that the surface area of a baby's head is significantly larger. Then it said that VERMEER Cosmolife calculates daily exposure to baby shampoo using the surface area in an adult. Shouldn't we, though, be using the surface area that the EPA has assumed? Although here it's --

DR. KLAASSEN: One probably should use the surface area of the child rather than the adult. Probably, in the end, it won't make that much difference.

DR. BELSITO: Okay. Then, looking at this entire document, if we knew impurities in the copper gluconate that was used in cosmetics, we could argue that it's GRAS, so we're not concerned about its systemic toxicity which, regardless, is okay. Although the genotox, I think, is a little weak. We also don't have dermal irritation and sensitization.

DR. RETTIE: Right.

DR. BELSITO: I would say that this document is insufficient for impurities, dermal sensitization, and irritation at concentration of use -- and ocular irritation, if available. Otherwise, the systemic endpoints look fine. Curt? Paul?

DR. SNYDER: I agree with the insufficient data announcement at this stage of the document.

DR. BELSITO: Okay. Curt?

DR. KLAASSEN: Yeah, I second that.

DR. BELSITO: Allan?

DR. RETTIE: Yeah, impurities, dermal irritation, and sensitization and ocular irritation seem to cover it.

DR. BELSITO: Okie doke. Any other comments?

MS. FIUME: Just the question about often -- I think it's particularly in ECHA -- there are those QSAR predictions. What are -- do the Panel find -- do you find those useful? Do you want those included? How would you like the writers to handle those

QSAR predictions, an example being under Dermal Irritation and Sensitization on PDF page 20? It's the very bottom paragraph under Dermal Irritation and Sensitization. It's discussing REACH's use of QSAR predictions.

DR. BELSITO: Right. I mean, here I think it's hard to estimate. It says, "...predicts copper gluconate would produce an irritation index of 2.26 in rabbit skin." Then another one with predicting the EC3 in an LLNA of 5.08 copper gluconate. First of all, we don't know what the level of copper that would induce that level of irritation. Is it assume that it's a hundred percent pure copper would cause an irritation index of 2.26? I don't know. Yeah, I think QSARs are nice. To me, just to use them, an In Silico model, to predict irritation and sensitization is not adequate.

MS. FIUME: Do you want these data to remain in the report? Or do you want them removed?

DR. BELSITO: I mean, they can remain in the report for now.

MS. FIUME: Okay.

DR. BELSITO: I would like to see some data on irritation and sensitization at concentrations of use. That --

DR. KLAASSEN: You're talk -- oh, excuse me.

DR. BELSITO: Pardon?

DR. KLAASSEN: I'm lost here. You're talking about page 20?

MS. FIUME: Yes.

DR. BELSITO: Yes, page 20, dermal irritation and sensitization are In Silico predictions --

DR. KLAASSEN: Oh.

DR. BELSITO: -- using a QSAR model.

DR. KLAASSEN: Okay. I see it.

DR. BELSITO: I'm basically saying that for irritation and sensitization I don't think In Silico models are adequate. I mean, I'd take an In Vitro model if they want to give me a DPRA, KeratinoSens, and LuSens or h-CLAT or, you know, but QSAR I have a little trouble with.

MS. FIUME: Yeah. These are just starting to appear in the ECHA dossiers. We're not sure how to use them. We're presenting them, but, again, we didn't know if the Panel even wants them in the report or not.

DR. BELSITO: I think if -- like, take sensitization. If we were relying simply on In Vitro tools, which are still only recently developed, and it would add weight of evidence to a prediction of a sensitizer or a non-sensitizer along with the In Vitro, I'd like to see it. If we have In Vivo data, then I think I really don't care what QSAR says.

MS. FIUME: Okay.

DR. BELSITO: I think it's like everything else. It's a case-by-case basis.

MS. FIUME: It seems to be going that way anymore.

DR. BELSITO: Yeah. Anything else on copper? If not, we're going to move to *t*-Butyl Alcohol.

Cohen Team – March 28, 2024

DR. COHEN: All right. Copper Gluconate. Are we ready to move onto the next? So, copper gluconate, this is a draft report on the safety assessment of copper gluconate and it's the first time we're reviewing this. The Panel had previously reviewed the safety of gluconic acid, potassium gluconate, and sodium gluconate in 2019 with a published conclusion of safe as used. Additionally, several QSAR models were described by the European Chemicals Agency dossiers for these endpoints, and they've been included in this report the CIR's performed an exposure assessment which has been referenced in many of the other discussions we've had in this section.

We acknowledge the second wave data and I think we agree with those suggestions, but we'll discuss them. This functions in cosmetics as a skin conditioning agent. The ingredients are generally regarded as safe so they GRAS as a direct human food ingredient. We have method of manufacturing and impurities. We have 2023 VCRP data of copper gluconate being used in 170 reported uses with 140 as leave on. And we have maximum concentration of 0.2 percent in baby shampoo and 0.006 percent in an eyeliner near the eye and we have a QSAR model.

So, I'll just open it up for discussion. Who wants to start? Tom, you want to lead us?

DR. SLAGA: Yeah. Very nice summary. Obviously, related compounds other salts of gluconate were found safe, and this particular copper version is GRAS. It's found in food -- put in foods for protection and also, we have, some data also supporting that it's -- number one, it's not genotoxic and I have some concerns about use with babies and around the eyes and I

would assume that it's really not an irritant to the eye, hopefully. Overall, I could push to go for safe as used based on even some of the calculations in the report.

DR. COHEN: I will round the bend and then reopen to discussion. David?

DR. ROSS: Yeah. I mean, it's quite interesting the highest concentration -- getting back to our comments about highest concentration but the max concentration was baby shampoo which I think is a little bit unusual for us. I found the exposure assessment very helpful and persuasive. The RDAs, adults and babies were 900 and 340 micrograms per day respectively. I think we're getting up to a body lotion exposure of about 70 micrograms. It's orders of magnitude less than the RFD, so I think that was leading me towards what Tom was saying is safe as used in present practices.

I think the major issue are these predictive QSARs for the NOAELs which we talked about in the previous two documents. It's particularly a concern for dermal and ocular where we have no data whatsoever. And I'd like to see -- I guess in a perfect world I would've liked to have seen some data of a molecular nature on ocular and dermal, skin cells, or corneal cells or something like that which gives us a little bit of reassurance, and a bit more confidence in these endpoints.

The RDA issue gives us a lot of confidence but what do we know about potential skin and ocular irritation. So, I wanted your comments on that, particularly David and Wilma.

DR. COHEN: Yeah.

DR. BERGFELD: The GRAS.

DR. COHEN: Susan, why don't you make your comments and then we'll come around and process everyone's statements.

DR. TILTON: Okay. So, I agree with Tom and David. I mean, in terms of the QSAR predictions, so those for repeat dose and reproductive and developmental. There was other toxicity data; there were other DART studies in which there weren't significant effects observed for reproductive effects and developmental endpoints and offspring. So, the QSAR predictions were pretty consistent with the other data that was being presented.

Unfortunately, as David mentioned there really were no other irritation or sensitization studies to rely on and the predictions ranged into the moderate and mild irritation, particularly around the eye. I think we're early enough with this report that we could request other dermal irritation or sensitization studies and see if there's data out there.

I mean, I tentatively agree, though, with safe as used.

DR. COHEN: So, the one issue to me is the irritation/sensitization and copper is a known sensitizer topically. It does have some correlation with nickel allergy which is the most common patch tested allergen probably in the world. And there is data on contact dermatitis to copper and there's data on patch testing down to 0.2 percent eliciting positive patch tests. To me, it wasn't the gluconate; it was the metal. The metals in contact dermatitis are always the issue and so I think we go for an IDA for irritation and sensitization at max concentration.

DR. ROSS: Yeah, I agree with that. I feel better with having some data to back that up rather than just the predictive NOAELs.

DR. COHEN: Yeah. We have, really nothing in human skin here that -- and there's plenty of reports out there, right, and I'll include them in my data feedback. But there's at least two articles I pulled. One from 2014, one from 2001 that goes through copper hypersensitivity reactions. They're in IUDs and have caused issues in some people. So, there's enough out there that we should ask for this. It's a rare sensitizer but it's well described.

DR. ROSS: I think I pulled the same papers. They described them as a weak sensitizer relative to the metals but (audio skip).

DR. COHEN: Right. And it's not like the, I mean, often the patch testing concentrations are two and five percent, but one of the studies took copper sensitive patients and patch tested them down to 0.2 percent and in some people, they were able to elicit reactions. So, it's not like -- we're right where the max uses in the baby shampoo, right? So, I think it behooves us to have that.

DR. EISENMANN: So, if I'm hearing you correctly, you're okay with data on other copper compounds on sensitization and irritation, correct? But I don't think they patch test with copper gluconate, they'd probably use copper sulfate or something like that.

DR. COHEN: Yes.

DR. EISENMANN: Okay.

DR. COHEN: Yes, but so the question is, I ran into the same issue in my head. Right, it's usually copper sulfate. There might've been one other one. But the question is I'm not sure, maybe Susan you'd know, of the valances for copper differ in copper sulfate than copper gluconate, because the valance of the metal is going to make a difference.

DR. ROSS: (Audio skip) copper gluconate and copper sulfate, they should be plus two not plus one.

DR. TILTON: Yeah, I would agree.

DR. COHEN: I think copper sulfate can exist in two different forms, right?

DR. ROSS: Copper can, it's plus two and a plus one. Yeah. Copper can.

DR. COHEN: Yeah. So, if copper sulfate can be a plus two and copper gluconate's a plus two then, yeah, I think we can -- I mean, the Belsito team might have a different opinion about it but usually it's the valence of the metal that's been predicting sensitivity like hexavalent chromium being very sensitizing. We've trivalent chromium not very sensitizing. So, if we're in the same valence state, yeah, I'd still like to see something. I'm open to hearing more. But just because they didn't patch test to it doesn't mean -- it's just the most readily available thing that they had.

DR. ROSS: I'm not sure about the valence argument, but I think they certainly need more. I agree with you.

DR. COHEN: Any other discussions on it? Wilma, did you have any other?

DR. BERGFELD: No, I thought that was good discussion and there's no reason not to call for the -- I think you want human, though, if you can get it, don't you, for irritation and sensitization?

DR. COHEN: Yeah, HRIPT.

DR. BERGFELD: Mm-hmm.

DR. ROSS: I mean, with the eye, you could ask for a molecular -- you can ask for corneal cells or whatever.

DR. COHEN: And an eye. Okay.

DR. ROSS: We had a few (audio skip) dossiers, human corneal cells that were used that'd be skin, I think, that were used in those.

DR. COHEN: Right. And if the HRIPT isn't showing any signaling even early on from those assaults then that gives us a little bit extra if we have non in vivo assessment like the corneal cells. Okay, we can move on to t-Butyl Alcohol?

Full Panel – March 29, 2024

DR. BELSITO: This is the first time that we're looking at this safety assessment. The SLR was announced in December of 2023. We previously reviewed the safety of gluconic acid, potassium gluconate, and sodium gluconate in 2019. Final Report was published with a conclusion that those ingredients were safe in the present practices of use and concentration in cosmetic described in this report.

Copper as we all know is an essential element, so much of the systemic toxicity or all of the systemic toxicity was really pretty much cleared by that. However, we only received information on the impurities on food-grade copper, so we don't have information on the impurities that might be present in copper gluconate. Nor do we have dermal irritation and sensitization or ocular irritation at concentrations of use. So, while we thought the systemic endpoints were okay, we're going out with insufficient for impurities, dermal irritation and sensitization at concentration of use, and ocular irritation if available.

DR. BERGFELD: Is there a second or a comment?

DR. COHEN: Second.

DR. BERGFELD: Any comment?

DR. COHEN: And Don had perfectly reviewed our needs.

DR. BERGFELD: Any comment about the copper sensitization? And, we're going to wait.

DR. COHEN: Well, that's why we're asking for irritation and sensitization, then, you know, Don, there's plenty of reports of copper contact dermatitis and even patch testing down to max use in sensitized people. But, that's neither here or there, we got the IDA out.

DR. BERGFELD: Okay. So it's been proposed and the comments have been made on presenting this as an IDA with the insufficiencies as noted. Those against? Abstaining? Unanimously approved to send this out as an IDA with the impurities and irritation, and sensitivities and ocular concerns data request. Okay, moving on to *t*-Butyl Alcohol, Dr. Cohen.

Safety Assessment of Copper Gluconate as Used in Cosmetics

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ABBREVIATIONS

ALT	alanine aminotransferase
APP	amyloid precursor protein
AUC	area-under-the-curve
BBN	*N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine
CAS	Chemical Abstracts Service
c-fos	protein c-Fos
CIR	Cosmetic Ingredient Review
CLP	Classification, Labelling, and Packaging regulation
C_{max}	concentration maximum
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
CTR1	copper transporter 1
DEN	*N*-nitrosodiethylamine
DHPN	2,2'-dihydroxy-di-*n*-propylnitrosamine
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
DMH	1,2-dimethylhydrazine
DMSO	dimethyl sulfoxide
DMT1	divalent metal transporter 1
DNA	deoxyribonucleic acid
ECHA	European Chemicals Agency
EC3	effective concentration to induce a 3-fold increase in local lymph node proliferative activity
ET_{50}	time for the test article to reduce the viability of the skin to 50%
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
Gadd45α	growth arrest and DNA damage inducible alpha
GGT	gamma glutamyl transpeptidase
GHS	Globally Harmonized System
GRAS	generally recognized as safe
GST-P	glutathione S-transferase placental form
HGF	hepatocyte growth factor
HRIPT	human repeated insult patch test
IL-1α	interleukin 1-alpha
INCHEM	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect-level
MMAS	modified maximum average score
MNU	*N*-methylnitrosourea
MRL	minimal risk level
mRNA	messenger RNA
MT1a	metallothionein 1a
MT2a	metallothionein 2a
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NA	not applicable
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
NOAEL	no-observed-adverse-effect-level
Nos2	nitric oxide synthase
NoG	Notes of Guidance
NR	not reported
OECD	Organisation for Economic Co-operation and Development
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
p21	tumor protein p21
p53	tumor protein p53
Panel	Expert Panel for Cosmetic Ingredient Safety
PDII	primary dermal irritation index
QSAR	quantitative-structure activity relationship
RDA	recommended daily allowance
REACH	Registration, Evaluation, Authorisation, and Restriction of Chemicals
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dose
STOT RE	specific target organ toxicity, repeated exposure

$t_{1/2}$	half-life
TG	test guideline
TGF-β	transforming growth factor-β
TNF-α	tumor necrosis factor alpha
TUL	tolerable upper limit
US	United States
USP	US Pharmacopeia
VCRP	Voluntary Cosmetic Registration Program

DRAFT ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Copper Gluconate, which is reported to function in cosmetics as a skin-conditioning agent. The Panel reviewed all relevant data and concluded…[to be determined].

INTRODUCTION

This assessment reviews the safety of Copper Gluconate as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), this ingredient is reported to function in cosmetics as a skin-conditioning agent.[1]

In 2019, the Panel published a final report that reviewed the safety of gluconic acid, potassium gluconate, and sodium gluconate, with the conclusion that these ingredients are safe in the present practices of use and concentration in cosmetics described in the safety assessment.[2] The full report can be accessed on the Cosmetic Ingredient Review (CIR) website: (https://cir-reports.cir-safety.org/).

The ingredient reviewed in this safety assessment is generally recognized as safe (GRAS) as a direct human food ingredient and as a nutrient or dietary supplement used in animal drugs, feeds, and related products; hence, daily exposure from food use would result in much larger systemic exposures than those from use in cosmetic products. Thus, the primary focus of the safety assessment of this ingredient as used in cosmetics is on the potential for local effects from topical exposure.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted August 2024. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Some of the data included in this safety assessment were found on an International Programme on Chemical Safety (INCHEM) Joint FAO/WHO Expert Committee on Food Additives (JECFA) webpage and the European Chemicals Agency (ECHA) website.[3,4] Please note that these sources provide summaries of information generated by industry, and it is those summary data that are presented in this safety assessment when these sources are cited.

CHEMISTRY

Definition and Structure

Copper Gluconate (CAS No. 527-09-3) is the copper salt of gluconic acid that conforms to the structure depicted in Figure 1.[1]

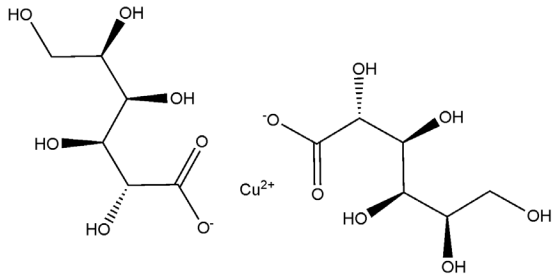

Figure 1. Copper Gluconate

Chemical Properties

Copper Gluconate is a light blue to bluish-green or green solid or crystalline, odorless powder that has a formula weight of 453.9 g/mol (compared to 63.55 g/mol atomic weight of copper) and an estimated log K_{ow} of - 2.98.[4-8] Additionally, Copper Gluconate has a density of 1.78 g/ml and is soluble in water; although slightly soluble in alcohol, it is insoluble in acetone, ether, and other organic solvents. The chemical properties of Copper Gluconate are further outlined in Table 1.

Method of Manufacture

The following are general methods of manufacture, and it is unknown whether these are utilized in the manufacture of Copper Gluconate as a cosmetic ingredient. In one method, a 1.0 M aqueous solution (6 ml) of gluconic acid (0.006 mol) is added to a suspension of copper hydroxide (0.003 mol) in 5 ml of distilled water.[6] The mixture is stirred at 75°C and monitored by infrared spectroscopy; the reaction is conducted until the absorption band for the carboxylic group of gluconic acid is no longer detectable. The solvent is evaporated on a rotary evaporator at 65 - 75°C, at a residual pressure of 10 - 20 mmHg, and the resulting residue is dried in a desiccator. According to 21CFR184.1260, Copper Gluconate is prepared by reacting gluconic acid solutions with cupric oxide or basic cupric carbonate.

Impurities

According to a supplier, specifications for food-grade Copper Gluconate powder included 98 – 102 % purity, with a 1% maximum limit for reducing substances.[8,9] Results from a certificate of analysis for a food-grade, US Pharmacopeia (USP)Copper Gluconate powder demonstrated a purity of 100.2%, copper content of 14%, reducing substances content of 0.21%, < 0.07% chloride and < 0.05% sulfate (both below maximum limits), 0.10 ppm arsenic (3 ppm maximum limit), 0.02 ppm lead (5 ppm maximum limit), a lack of coliform presence, and aerobic plate count and yeast and mold counts that were below specification limits (< 1000 cfu/g and < 100 cfu/g, respectively).[8,10] In an elemental impurity analysis of a USP Copper Gluconate powder, none of the tested elements were present above typical threshold values.[11] According to specifications provided by another supplier, the presence of cadmium, chromium, mercury, selenium, and thallium (each < 0.1 ppm), arsenic, cobalt, lithium, molybdenum, and vanadium (each < 1 ppm), antimony, barium, and lead (each < 2 ppm), and nickel (< 5 ppm) in Copper Gluconate would be unlikely and minimal.[12] Additionally, specifications for food-grade Copper Gluconate include an acceptance criteria of no more than 5 mg/kg lead in a 1 g sample of Copper Gluconate.[9]

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics and does not cover its use in airbrush delivery systems. Data were submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP survey data, Copper Gluconate has 170 reported uses, 140 of which are in leave-on formulations (Table 2).[13] The results of the concentration of use survey conducted by the Council in 2024 indicate that the maximum reported concentration of use for Copper Gluconate in a leave-on formulation is up at 0.008% in non-spray night products; overall, the highest maximum reported concentration of use is 0.36% in other oral hygiene products.[14]

Several uses in products applied near the eye (at up to 0.006% in eyeliners) and in products that can result in incidental ingestion have been reported (e.g., it has 4 reported uses in mouthwashes and breath fresheners, 2 reported uses in lipsticks; and is used at 0.36% in other oral hygiene products; other concentrations not provided). Copper Gluconate is reported to be used in baby shampoos and baby lotions, oils, powders, or creams at 0.00008%.

Copper Gluconate is also reported to be used in face powder formulations (concentration not provided) and could possibly be inhaled. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetics would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

Although products containing this ingredient may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of this ingredient (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

Copper Gluconate is not restricted from use in any way under the rules governing cosmetic products in the European Union (EU).[15]

Non-Cosmetic

According to the US National Institutes of Health Office of Dietary Supplements, copper is an essential mineral which is naturally present in the human body and in some foods; 900 µg is the recommended daily allowance (RDA) for adult copper intake.[16] The tolerable upper limit (TUL) for copper intake is 10,000 µg/d.[17]

As indicated in 21CFR184.1260, Copper Gluconate is affirmed as GRAS by the US FDA as a direct human food ingredient, which includes use in nutrient supplements and in infant formula, provided that levels do not exceed current good manufacturing practices. In addition, Copper Gluconate is also considered GRAS as a nutrient or dietary supplement used in animal drugs, feeds, and related products at a level not to exceed 0.005% (21CFR582.5260) and as a trace mineral added to animal feed (21CFR582.80), both in accordance with good manufacturing or feeding practices. According to 21CFR310.545, Copper

Gluconate has been present as an active ingredient in over-the-counter drug products for weight control; however, based on the currently available evidence, there is inadequate data to establish the safety or effectiveness of this use.

In the EU, copper and Copper Gluconate are categorized as mineral substances in Annex II of vitamin formulations and mineral substances which may be added to foods[18] and as minerals in Annex II of vitamin and mineral substances which may be used in the manufacture of food supplements;[19] listing in Annex II indicates the approved form for use in foods and food supplements. Additionally, Copper Gluconate is categorized as a mineral and is allowed in all 4 categories of food intended for infants and young children (i.e., infant formula and follow on formula; processed cereal-based food and baby food; food for special medical purposes; and total diet replacement for weight control).[20]

TOXICOKINETIC STUDIES

Oral

Groups of 449-d-old male C57BL/6J mice (5/group) were administered 0.005 M Copper Gluconate in drinking water for 92 d.[21] The accumulation of copper (dry weight) in the liver, kidney, brain, and heart of the test animals was compared to that of controls (drinking water). There was a statistically significant increase in copper accumulation in the livers of Copper Gluconate-fed mice, compared to controls (28.6 vs. 13.5 ng/mg). Differences between the amount of copper found in the kidney, brain, and heart of Copper Gluconate-fed mice and control mice were not statistically significant. In a related study, groups of 5 – 7 male C57BL/6J mice were administered 0.005 M Copper Gluconate in drinking water for 104 d, starting from various ages (64, 302, and 540 d of age). The accumulation of copper (dry weight) in the liver and kidney of Copper Gluconate-fed mice and controls (drinking water) was compared at the end of the experiment. The difference between copper accumulation in the liver of Copper Gluconate-fed mice and control mice was statistically significant in all 3 age groups; no statistically significant differences were observed in the amount of copper found in the kidneys of Copper Gluconate-fed mice (in all 3 age groups) compared to controls.

In a biodistribution study of copper (administered as Copper Gluconate), male Wistar rats (total number not specified) received a single dose of 79.5 mg/kg Copper Gluconate, dissolved in deionized water, via gavage, and were observed for up to 168 h prior to necropsy (rats were killed at 0.08, 0.17, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 12, 24, 48, 72, and 168 h).[22] Blood samples, brain tissue (striatum and midbrain), and liver samples were collected at each time point (n = 4 – 6). Controls received deionized water and were killed immediately after treatment; copper concentration in control blood and tissue samples was considered baseline. A plasma copper concentration maximum (C_{max}) value of 1.94 ±0.28 µg/ml was observed 1.5 h post-treatment, which was 73.1% higher than the baseline concentration ($p < 0.01$). Copper plasma concentration returned to baseline 72 h after treatment and the half-life ($t_{1/2}$)and area-under-the-curve (AUC) values were about 1.79 h and 2.48 ± 0.36 µg/ml*h, respectively. The C_{max} for copper distribution in the striatum tissue of Copper Gluconate-treated rats was 2.93 ± 0.21 µg copper/g of wet tissue at 0.25 h post-treatment (49.9% higher than baseline values) which returned to baseline after 168 h. A 27.6% increase in copper concentration (3.87 ± 0.25 µg copper/g of wet tissue) was observed in the midbrain of treated rats at 0.25 h post-treatment, however, no significant differences in copper concentration in the midbrain tissue of treated and control rats were observed. The C_{max} of copper in the liver of Copper Gluconate-treated rats was arrived at 12 h post-administration and was 391% higher than baseline (23.25 ± 1.75 vs. 4.735 ± 0.29 µg copper/g of wet tissue). Elimination or redistribution of copper found in the liver was observed 24 h post-administration. The area-under-the-curve $(AUC)_{0-168\ h}$ value for liver copper concentration was about 200 times greater than the AUC value for plasma copper concentration (494.8 ± 47.22 vs. 2.48 ± 0.36 µg/ml*h).

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Details on the acute dermal (computational) and oral toxicity studies summarized below can be found in Table 3.

According to a quantitative structure-activity relationship (QSAR) model described in an ECHA dossier, the acute dermal LD_{50} for Copper Gluconate was predicted to be 2130 mg/kg bw in rats.[4] In an acute oral administration study, male Wistar rats (4 – 6/group) were administered a single oral dose of 79.5, 156, or 312 mg/kg Copper Gluconate, in deionized water.[22] The survival rate of rats in the 312 mg/kg group was 31%. No animals from the 79.5 and 156 mg/kg groups died and no significant differences in weight gain or activity levels of the hepatic enzymes gamma glutamyl transpeptidase (GGT) or alanine aminotransferase (ALT) were observed after 7 d of exposure compared to the control group. Male and female Wistar rats (5/sex/group) were administered a single dose of up to 3200 mg/kg Copper Gluconate, in water, in an acute oral toxicity study.[4] Five out of 10 of the animals from the 1800 mg/group died within 48 h of exposure, 8 out of 10 animals in the 2400 mg/group died within 48 h of exposure, and all 10 animals from the 3200 mg/kg group died within 24 h of exposure. The acute oral LD_{50} was determined to be 1709 mg/kg bw for both sexes.

Short-Term and Chronic Toxicity Studies

Details on the oral short-term and chronic oral toxicity studies and a computational study to predict short-term oral toxicity summarized below can be found in Table 4.

Groups of 5 male Fischer 344 rats were administered 0, 0.001, 0.03, or 0.6% (equivalent to 0, 10, 300, or 6000 ppm, respectively) Copper Gluconate in the diet for 2 wk, in a short-term oral toxicity study.[23] No differences in final body weight, liver

weight, food consumption, or gross or histological changes in the liver were observed in the treated animals, compared to controls. Upon performing gene expression analysis in the liver, hepatic messenger ribonucleic acid (mRNA) expression of metallothionein 1a (*Mt1a*; a metal metabolism-related gene) and growth arrest and deoxyribonucleic acid (DNA) damage inducible alpha (*Gadd45α*; an apoptosis-related gene) were significantly increased in the 0.6% Copper Gluconate group and *p21* (tumor protein p21; an apoptosis-related gene) expression was significantly increased in the 0.03% and 0.6% dose groups. Expression levels of *p53* (tumor protein p53; an apoptosis-related gene) and inflammation-related genes, such as *TNF*-α (tumor necrosis factor alpha), *IL*- 1α (interleukin 1-alpha), *Nos2* (nitric oxide synthase 2), and *c-fos* (protein c-Fos; a proto-oncogene) were not affected.

No adverse effects were noted in food consumption, body weight gain, urinalysis, or gross and microscopic examination of tissues and organs in male and female rats that were administered at dietary levels of 0.006 or 0.06% Copper Gluconate (mean daily consumption of 3.46 or 34.9 mg/kg/d, respectively) in the diet for 24 wk.[24] Copper content was elevated in the kidneys of animals fed the diet containing 0.06% Copper Gluconate. In a chronic oral toxicity study, groups of 25 rats were administered 1.14% Copper Gluconate in the diet for up to 44 wk.[3,25] Significant growth retardation was discernible at 26 wk compared to controls, and over 80% of the animals died between week 17 and week 35. Upon necropsy, hypertrophied uteri, ovaries, seminal vesicles and hypertrophied stomachs, occasional ulcers, bloody mucus in the intestinal tract, and bronzed kidneys and livers were observed; chronic exposure to 1.14% Copper Gluconate in the diet was considered toxic. Groups of 6 male and 6 female Beagle dogs were administered 0.012, 0.06, or 0.24% Copper Gluconate in the diet (equivalent to 3, 15, or 60 mg/kg/d, respectively) for up to 1 yr.[3,25] Accumulation of copper was seen in the liver, kidneys, and spleen of animals in the 0.24% group; minimal liver function was observed in 1 out of 12 dogs in the 0.24% group after 1 yr of dosing, which was reversible within a 12-wk withdrawal period. No other test-article related effects were observed. Male C57BL/6J mice (number not specified) received 0.0005, 0.001, or 0.005 M Copper Gluconate in drinking water over the animal lifetime.[21] The survival curve and lifespan were significantly reduced by 11.8, 14.7 and 14.4% in the 0.0005, 0.001 and 0.005 M groups, respectively, indicating the absence of a dose-response relationship for survival. The effect of administering copper to adult Capuchin monkeys (2/sex; 7.5 mg/d) and copper as Copper Gluconate to young Capuchin monkeys (2/sex; 5.5 mg/d), in the diet, was evaluated in a 156-wk (3-yr) oral toxicity study.[26] No differences in food intake, body weight, or weight gain by age or time of exposure were observed in treated adult and young Capuchin monkeys, compared to age-matched controls. After 24 mo, Ki67 and MT1 protein levels were significantly greater in the liver tissue of treated adult and young monkeys. Upon further analysis of adult liver tissue after 36 mo, hepatic mRNA expression of proteins related to inflammation and hepatic response to injury (nuclear factor kappa-light-chain-enhancer of activated B cells (*NFκB*), hepatocyte growth factor (*HGF)*, and transforming growth factor-β (*TGFβ))*) were significantly greater in treated animals compared to controls, with no further evidence of clinical, hematological, or histological evidence of liver damage.

According to a QSAR model described in an ECHA dossier, the oral lowest-observed-adverse-effect-level (LOAEL) for Copper Gluconate in rats was predicted to be 94.7 mg/kg bw/d.[4] Based on this value and the Classification, Labelling, and Packaging (CLP) regulation, the specific target organ toxicity for repeated exposure-2 (STOT RE-2) designation, indicating presumed toxicity to specific organs with repeated exposure, was considered applicable.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Details on the oral developmental and reproductive toxicity studies and computational studies to predict such toxicity are summarized below and can be found in Table 5.

Groups of male albino rats (8/group) were used to examine the toxicological effects of Copper Gluconate upon oxidative biomarkers in testis tissue in a 90-d reproductive toxicity study.[27] The animals received 3.75, 7.5, or 15 mg/kg/d Copper Gluconate, via gavage; 2 control groups received either 1 ml of saline or 0.5 ml dimethyl sulfoxide (DMSO), via gavage, for the duration of the study. Treatment with Copper Gluconate did not significantly affect catalase levels but did significantly reduce glutathione and superoxide dismutase levels (at the medium and high dose). Additionally, malondialdehyde levels were also increased in treated rats, compared to controls; the study results are indicative of the development of oxidative stress in testes tissue. Female Swiss-Webster mice (20/group) and female albino Wistar rats (number/group not specified) received 0, 0.1, 3, or 30 mg/kg/d Copper Gluconate, via gavage, from day 6 to 14 of gestation, and from day 5 to 15 of gestation, respectively, in two separate developmental oral toxicity studies.[3,25] Neither embryotoxic nor teratogenic effects were observed in treated animals, compared to controls, in either study. In another oral developmental toxicity study, female Wistar rats (20/group) received up to 30 mg/kg/d Copper Gluconate, via gavage.[3,25] Female rats were dosed with Copper Gluconate 15 d prior to mating, during gestation, and for 21 d postpartum. Groups of treated females, from each dose group, were mated with untreated males. To assess the effects of Copper Gluconate on the male rat, 2 additional groups of males that were treated with 3 mg/kg/d Copper Gluconate 60 d prior to mating were mated with a group of untreated females or with a group of females that received the same 60-d pre-treatment. A third group of untreated males mated with untreated females served as controls. Male rat reproductive performance was not affected by Copper Gluconate administration. No significant differences were observed between the percentage of pregnancies, the number and distribution of embryos in each uterine horn, implantation sites, resorption sites, duration of gestation, mean number of fetuses and live pups per litter, litter size, stillborn and live born numbers, gross anomalies and mean weight per pup, compared to controls. Necropsy of dams and pups revealed a lack of visceral abnormalities. Thus, under the conditions of

the study, the researchers concluded that Copper Gluconate did not affect the reproductive performance of either male or female rats.

As described in an ECHA dossier, 2 separate models following the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) Guidance on QSARs and Grouping of Chemicals R.6 were used to predict the developmental and reproductive toxicity of Copper Gluconate in rats.[4] The no-observed-adverse-effect-level (NOAEL) of Copper Gluconate for oral reproductive toxicity in rats was predicted to be 318 mg/kg bw/d and the NOAEL of Copper Gluconate for oral developmental toxicity in rats was predicted to be 793 mg/kg bw/d.

GENOTOXICITY STUDIES

In Vitro

Copper Gluconate was tested at up to 1 mg/plate using *Salmonella typhimurium* strains TA97 and TA102 in an Ames test, according to Environmental Protection Agency (EPA) Office of Prevention, Pesticides, and Toxic Substances (OPPTS) 870.5265.[4] The test article was not genotoxic, with or without metabolic activation. Additionally, Copper Gluconate was evaluated for mutagenicity in various in vitro tests using *S. typhimurium* strains TA1535, TA1537, and TA1538, and *Saccharomyces cerevisiae* strain D4.[3,25] The test article was not considered mutagenic, with or without metabolic activation. No further details were provided.

Computational

QSAR model results predicting the genotoxic potential of Copper Gluconate were described in an ECHA dossier.[4] Using QSAR Toolbox 3.4.0.17, and based on REACH guidance R.6, Copper Gluconate was predicted to be non-genotoxic in an Ames test (with and without metabolic activation) and in a chromosome aberration test. Based on the expert rule-based system, Derek Nexus 6.3.0, Copper Gluconate exposure is not predicted to cause in vivo mutagenicity (*Mutagenicity in vivo* endpoint).[CIR staff]

CARCINOGENICITY STUDIES

Tumor Promotion

Five-wk-old male Fischer 344 rats (9 - 12/group) were given a single intraperitoneal injection of 200 mg/kg bw *N*-nitroso-diethylamine (DEN) as a carcinogenic initiator, and after 2 wk, received 0, 0.001, 0.03, or 0.6% (0, 10, 300, or 6000 mg/kg/d) Copper Gluconate in a basal diet for 6 wk, in a medium-term liver carcinogenicity bioassay.[23] Simultaneously, two additional groups which did not receive the nitrosamine injection prior were fed 0 or 0.6% Copper Gluconate in the diet. Numbers of glutathione *S*-transferase placental form (GST-P) positive lesions, single GST-P-positive hepatocytes, 8-oxoguanine-positive hepatocytes, and levels of cell proliferation and apoptosis in the liver were significantly increased in the 0.6% Copper Gluconate group, with and without nitrosamine pre-treatment. Furthermore, the hepatic mRNA expression of the metal metabolism-related gene *Mt1a*, the apoptosis-related genes *Gadd45α* and *p21*, the inflammation-related genes *TNF-α, IL-1α,* and *Nos2*, and *c-fos* were significantly increased in the 0.6% group, irrespective of nitrosamine treatment, while *p53* expression was significantly increased in the 0.03 and 0.6% Copper Gluconate groups which received the nitrosamine injection and in the 0.6% group which did not receive the nitrosamine injection. In the absence of the DEN treatment, animals treated with Copper Gluconate did not develop GST-P-positive lesions in the liver. While treatment with Copper Gluconate may have been associated with carcinogenic risk toward the liver at a high dose level (0.6%), the researchers indicated there is a considerably large safety margin for Copper Gluconate at the human relevant dose of 0.001 and 0.03% (the 0.001% dose nearly corresponds to the daily human intake of Copper Gluconate, as a food additive).

Groups of male Brl:Han Wistar rats (3 rats/group) were used to evaluate the toxicologic and carcinogenic risk of Copper Gluconate in a 13-wk medium-term multi-organ carcinogenesis assay.[28] Throughout the experiment, animals were fed a diet containing 0, 0.1, 0.3, 0.48, or 0.6% (equivalent to 0, 1000, 3000, 4800, or 6000 mg/kg/d, respectively) Copper Gluconate, or 1.2% (12,000 mg/kg/d; 1 animal) Copper Gluconate, while being exposed to multiple carcinogens. All animals received a single intraperitoneal administration of 100 mg/kg bw DEN followed by 4 intraperitoneal injections of 20 mg/kg bw *N*-methylnitrosourea (MNU) and 0.05% *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine (BBN), administered in drinking water, during the initial 2 wk. In the following 2 wk, the animals received 4 subcutaneous injections of 40 mg/kg bw 1,2-dimethylhydrazine (DMH) and 0.1% 2,2'-dihydroxy-di-*n*-propylnitrosamine (DHPN), in drinking water. The animals were killed and necropsied after 13 wk. Blood samples were taken from the abdominal aorta, urine samples were taken from the bladder, and major organs and tissues were removed; the liver was weighed and fixed for histopathological, histochemical, and immunohistochemical analyses. All animals survived until killed. Body weight and food consumption were similar between groups. Black stool was found in rats exposed to ≥ 0.3% Copper Gluconate. Copper levels in the serum, urine, and liver were significantly increased in animals dosed with ≥ 0.6% Copper Gluconate. Absolute and relative liver weights were similar among groups but appeared to increase in the 1 animal that received 1.2% Copper Gluconate. Livers were macroscopically and histologically normal in the groups dosed with ≤ 0.48%; slight or moderate granulomas were scattered in livers of animals in the 0.6% group. Copper accumulation and metallothionein induction were apparent at doses of ≥ 0.3% and ≥ 0.1% Copper Gluconate, respectively. Marked diffuse granulomas and hepatocellular necrosis were observed in the liver of the animal in the 1.2% Copper Gluconate group (1 rat in this group). Putative preneoplastic lesions appeared in the rat dosed with 1.2% Copper Gluconate and 8-hydroxydeoxyguanosine formation was

enhanced in the 0.6% group. The researchers indicated that under the current experimental conditions with co-exposure to multiple carcinogens, Copper Gluconate did not exert significant systemic toxicity, i.e., there were no differences in mean body weights among groups and in any treatment-related alternations in extrahepatic organs/tissues; however, it was noted that Copper Gluconate may cause toxic and carcinogenic risks towards the liver at high doses.

OTHER RELEVANT STUDIES

Nephrotoxicity

In a 90-d oral toxicity study examining the effects of Copper Gluconate on renal function, groups of 8 male albino Swiss rats were administered 3.75, 7.5, or 15 mg/kg Copper Gluconate, in saline, via gavage.[29] Controls received either 1 ml saline or 0.5 ml DMSO. Two animals per group were killed and blood samples were collected via cardiac puncture on days 30, 45, 60, and 90 for serum analysis. A statistically significant increase in urea, creatinine, sodium, and potassium levels was observed in renal serum obtained from treated animals, compared to controls. The results indicated development of renal failure and oral ingestion of the test article was considered nephrotoxic.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Details of the human dermal irritation and sensitization and computational studies described below can be found in Table 6.

A leave-on baby product formulation and a rinse-off adult product formulation, each containing 0.00008% Copper Gluconate (dose/unit area: 0.00004 mg/cm^2), were found to be non-irritating and non-sensitizing when applied neat in 2 separate human repeated insult patch tests (HRIPT), using 210 and 211 subjects, respectively.[30,31] A powder containing 0.1% Copper Gluconate (up to 0.038 mg/cm^2) was not irritating or sensitizing when applied in distilled water to 52 subjects in an HRIPT.[32] A rinse-off baby product formulation containing 0.2% Copper Gluconate (0.1 mg/cm^2) was also non-irritating and non-sensitizing when applied neat in an HRIPT using 217 subjects.[33] Based on QSAR models described in an ECHA dossier, Copper Gluconate was predicted to produce a primary dermal irritation index (PDII) of 2.26 in rabbit skin and 5.08% Copper Gluconate was predicted to be the effective concentration needed to induce a 3-fold increase in local lymph node proliferative activity (EC3) in a mouse skin model.[4] Based on an EC3 value > 2%, Copper Gluconate was classified according to Globally Harmonized System (GHS) criteria as having low to moderate skin-sensitizing potential (Skin Sensitizer Category 1B, under GHS category 1: substances that show a low to moderate frequency of occurrence in humans and/or low to moderate potency in animals and can be presumed to potentially produce significant sensitization in humans.[4,34]

OCULAR IRRITATION STUDIES

Details of the in vitro and computational ocular irritation studies described below can be found in Table 7.

The potential for a face cream containing 0.0025% Copper Gluconate to cause ocular irritation was evaluated in a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay using an in vitro tissue model.[35] Tissues were treated with the test article for up to 24 h. More than 24 h of treatment time was required to achieve a 50% reduction in tissue cell viability; the test article was classified as minimally or not irritating to eyes. Using QSAR prediction software (QSAR Toolbox 3.4.0.17) and the REACH guideline on QSAR, the modified maximum average score (MMAS) for Copper Gluconate was predicted to be 49.5 in rabbit eyes.[4] Copper Gluconate was predicted to be mildly toxic, considering that the maximum value for damage to the cornea, conjunctiva, and iris is 110. Based on GHS criteria, Copper Gluconate was considered to be a potential mild irritant to the eyes (Category 2B).

CLINICAL STUDIES

Oral Supplementation

The effect of copper supplementation, in the form of Copper Gluconate, was evaluated in a 12-wk, double-blind, randomized study.[36] Seven subjects (3 men and 4 women) received either a 5 mg capsule of Copper Gluconate or placebo twice a day. Blood, serum, urine, and hair samples were collected at the beginning of the study, 6 wk after supplementation, and at the end of the 12 wk. Copper, zinc, and magnesium levels were determined in all the samples; no significant changes were observed in serum, urine, or hair for the study duration. No significant changes in hematocrit, mean corpuscular volume, serum cholesterol, triglyceride, glutamic-oxaloacetic transaminase, alkaline phosphatase, gamma-glutamyl transferase, or lactate dehydrogenase levels were observed in treated subjects. Serum potassium levels did change from a mean of 4.3 mEq/l to 4 mEq/l ($p < 0.05$). The incidence of nausea, diarrhea, and heartburn was the same in both treated subjects and controls.

EXPOSURE ASSESSMENT

Copper is an essential mineral, which is naturally present in some foods and can also be taken as a dietary supplement. As a food additive, Copper Gluconate may serve as a nutritional supplement for copper.[16] The daily copper intake needed to fulfill the nutritional needs averages 900 µg/d for adults (aged 19+ yr) and 340 µg/d for babies (aged 1-3 yr). Additionally, the highest daily

intake that is unlikely to lead to adverse health effects is set at 10,000 µg/d for adults (aged 19+ yr) and 1000 µg/d for babies (aged 1-3 yr).

CIR staff applied exposure parameters identified from literature and the in silico tool VERMEER Cosmolife (previously named SpheraCosmolife)[37] to estimate the daily exposure to copper that results from the highest use concentration of Copper Gluconate (e.g., 0.36% in mouthwash) and from exposure to other product categories (e.g., 0.1% in make-up remover and 0.008% in body lotion).

i) Copper Gluconate at 0.36% in oral hygiene products (e.g., mouthwash)

The following parameters are retrieved from a 2008 dermal sensitization risk assessment[38]:
Estimated amount applied of mouthwash per application: 10 g (or 10,000mg)
Frequency of application: 3/d
Retention factor: 0.01
Type of exposure: incidental ingestion
Relative daily exposure of mouthwash: 10,000 mg/d × 3 (application times) × 0.01 (retention factor) = 300 mg/d
Exposure to Copper Gluconate as used in mouthwash: 300 mg/d × 0.36% (use concentration) = 1.08 mg/d

The proportion of copper in Copper Gluconate is approximately 14%; therefore, daily exposure to copper from Copper Gluconate in mouthwash: 1.08 mg/d × 14% = 0.1512 mg/d = 151.2 µg/d
Systemic exposure dose (SED) with 100% absorption (oral absorption): 151.2 µg/d

ii) Copper Gluconate at 0.1% in skin cleansing preparations (e.g., make-up remover)

The following parameters are retrieved from the SCCS NoG.[39]
Estimated daily amount of make-up remover applied: 5 g/d = 5000 mg/d
Retention factor: 0.1
Type of exposure: rinse-off
Surface area for application: 565 cm^2
Relative daily exposure of make-up remover: 5000 mg/d × 0.1 (retention factor) = 500 mg/d
Exposure to Copper Gluconate as used in make-up remover: 500 mg/d × 0.1% (use concentration) = 0.5 mg/d
Daily exposure to copper from Copper Gluconate in make-up remover: 0.5 mg/d × 14% = 0.07 mg/d = 70 µg/d
SED with 100% absorption (oral absorption): 70 µg/d
Skin surface exposure: 70 µg/d ÷ 565 cm^2 = 0.124 µg/cm^2/d

iii) Copper Gluconate at 0.008% in non-spray night products (e.g., body lotion, leave-on)

The following parameters are retrieved from the SCCS NoG.[39]
Estimated daily amount of body lotion applied: 7.82 g/d = 7820 mg/d
Retention factor: 1.0
Type of exposure: leave-on
Surface area for application: 15,670 cm^2
Relative daily exposure of body lotion: 7820 mg/d × 1.0 (retention factor) = 7820 mg/d
Exposure to Copper Gluconate as used in body lotion: 7820 mg/d × 0.008% (use concentration) = 0.6256 mg/d
Daily exposure to copper from Copper Gluconate in body lotion: 0.6256 mg/d × 14% = 0.0876 mg/d = 87.6 µg/d
SED with 100% absorption (oral absorption): 87.6 µg/d
Skin surface exposure: 87.6 µg/d ÷ 15,670 cm^2 = 0.0056 µg/cm^2/d

The exposure assessment indicates that the daily exposure to copper from Copper Gluconate in mouthwash, make-up removers, and body lotions does not exceed 151.2 µg/d, 70 µg/d, and 87.6 µg/d, respectively. These exposure levels are substantially below the RDA of 900 µg/d for adults or 340 µg/d for babies (1-3 yr), as well as the TUL of 10,000 µg/d for adults or 1000 µg/d for babies.

SUMMARY

The safety of Copper Gluconate is reviewed in this safety assessment. As per the *Dictionary*, this ingredient is reported to function as a skin conditioning agent in cosmetics. According to 2023 VCRP and 2024 Council survey data, Copper Gluconate is

reported to be used in 170 formulations, 140 of which are leave-ons. The highest reported concentration of use is at 0.36% in other oral hygiene products; the highest reported concentration in a leave-on formulation is at up to 0.008% in non-spray night products. Copper Gluconate is also reported to be used in baby shampoos and baby lotions, oil, powders or creams at 0.00008%. Copper is an essential mineral which is naturally found in the human body and in foods; the RDA and TUL for adult copper intake is 900 µg and 10,000 µg/d, respectively. Notably, Copper Gluconate is considered GRAS as a direct food substance for human consumption, which includes use in nutrient supplements and in infant formula.

Groups of male C57BL/6J mice (5/group) were administered 0.005 M Copper Gluconate in drinking water for 92 d. A statistically significant increase in copper accumulation in the livers of Copper Gluconate-fed mice was observed, compared to controls. Differences between the amount of copper found in the kidney, brain, and heart of Copper Gluconate-fed mice, compared to controls (drinking water) were not statistically significant. Groups of male C57BL/6J mice (5 -7/group) were administered 0.005 M Copper Gluconate in drinking water for 104 d, starting from 64, 302, and 540 days of age. The difference between copper accumulation in the liver of Copper Gluconate-fed mice and control mice was statistically significant in all 3 age groups; no statistically significant differences were observed in copper accumulation in the kidneys (in all 3 age groups), compared to controls. In a biodistribution study of copper, male Wistar rats received a single dose of 79.5 mg/kg Copper Gluconate, dissolved in deionized water, via gavage and were observed for up to 168 h. A C_{max} of 2.93 ± 0.21 µg copper/g in brain striatum tissue at 0.25 h returned to baseline after 168 h. No significant differences in copper concentration in the midbrain tissue of treated and control rats was observed. The C_{max} of copper in the Copper Gluconate-treated liver was 391% higher than baseline (elimination and redistribution of copper occurred 24 h after administration) and the AUC value for copper in the liver was about 200 times greater than the AUC for plasma copper concentration (494.8 ± 47.22 vs. 2.48 ± 0.36 µg/ml*h).

An acute dermal LD_{50} of 2130 mg/kg Copper Gluconate was predicted for rats, based on a QSAR model. No significant differences in weight gain or hepatic enzyme activity were observed in male Wistar rats that were administered a single oral dose of 79.5, 156, or 312 mg/kg Copper Gluconate. The survival rate of rats in the 312 mg/kg group was 31%; no animals in the 79.5 or 156 mg/kg groups died. Male and female Wistar rats received a single dose of 1800, 2400, or 3200 mg/kg bw Copper Gluconate, in water, via gavage, in another acute oral toxicity study. Five out of 10 of the animals from the 1800 mg/group died within 48 h of exposure, 8 out of 10 animals in the 2400 mg/group died within 48 h of exposure, and all 10 animals from the 3200 were found dead within 24 h of dosing. The acute oral LD_{50} was determined to be 1709 mg/kg bw (males and females combined).

No differences in final body weight, liver weight, food consumption, or gross or histological changes were observed in male Fischer 344 rats (5/group) that were administered 0, 0.001, 0.03, or 0.6% Copper Gluconate in the diet for 2 wk in a short-term oral toxicity study. Hepatic mRNA expression of *Mt1a* and *Gadd45α* were significantly increased in the 0.6% group and *p21* expression was significantly increased in the 0.3 and 0.6% groups; other gene expression levels were unaffected.

Male and female rats that were administered 0.006 or 0.06% Copper Gluconate in the diet for 24 wk exhibited no adverse effects in food consumption, body weight gain, urine analysis, or gross or microscopic examination of tissues and organs; copper content was elevated in the kidneys of animals in the 0.06% Copper Gluconate group. Groups of 25 male and female rats received 1.14%Copper Gluconate in the diet for up to 44 wk in a chronic oral toxicity study. Significant growth retardation was discernable at 26 wk, compared to controls, and over 80% of the animals died by week 35. Hypertrophied uteri, ovaries, seminal vesicles and hypertrophied stomachs, occasional ulcers, bloody mucus in the intestinal tract, and bronzed kidneys and livers were observed upon necropsy; chronic exposure to 1.14% Copper Gluconate in the diet was considered toxic. Male and female Beagle dogs (6/sex/group) were administered 0.012, 0.06, or 0.24% Copper Gluconate, in the diet, for up to 1 yr; aside from copper accumulation in the liver, kidney, and spleen of animals in the 0.24% group, and reversible minimal liver function in 1 dog from the 0.24% group, no other test-article related effects were observed. The survival curve and lifespan of male C57BL/6J mice (number not specified) which received 0.0005, 0.001, or 0.005 M Copper Gluconate in drinking water during the lifetime were significantly reduced by up to 11.8, 14.7 and 14.4%, respectively, indicating the absence of a dose-response relationship for survival. No differences in food intake, body weight, or weight gain by age or time of exposure were observed in adult Capuchin monkeys (2/sex) that were fed up to 7.5 mg/d copper, and in young Capuchin monkeys (2/sex) fed up to 5.5 mg/d copper (as Copper Gluconate), in a 3-yr oral toxicity study. In the adult monkeys, the hepatic mRNA expression of proteins related to inflammation and hepatic response to injury (*NFκB*, *HGF*, and *TGFβ*) were significantly greater in treated animals compared to controls, with no further evidence of clinical, hematological, or histological evidence of liver damage. Using a QSAR model, the oral LOAEL for Copper Gluconate in rats was predicted to be 94.7 mg/kg bw/d; toxicity to specific organs with repeated exposure, as outlined in the specific target organ toxicity for repeated exposure-2 designation, was considered applicable.

Male albino rats (8/group) received 3.75, 7.5, or 15 mg/kg/d Copper Gluconate, via gavage, in a 90-d reproductive toxicity study. Oxidative biomarkers in rat testis tissue revealed that Copper Gluconate did not significantly affect catalase levels but did significantly reduce glutathione and superoxide dismutase levels (at the medium and high dose), while increasing malondialdehyde levels, compared to controls. These findings indicated the development of oxidative stress. In two separate developmental oral toxicity studies, neither embryotoxic nor teratogenic effects were observed in female Swiss-Webster mice (20/group) or female albino rats (number not specified) that received 0, 0.1, 3, or 30 mg/kg/d Copper Gluconate, via gavage, during gestation. Groups of female Wistar rats (20/group), mated with untreated males and males treated with 3 mg/kg/d Copper Gluconate (both 10/group), received up to 30 mg/kg/d Copper Gluconate in another developmental toxicity study. No significant differences were observed between the percentage of pregnancies, the number and distribution of embryos in each uterine horn, implantation sites, resorption

sites, duration of gestation, mean number of fetuses and live pups per litter, litter size, stillborn and live born numbers, gross anomalies and mean weight per pup, compared to controls. Under the conditions of this study, Copper Gluconate did not affect the reproductive performance of either male or female rats. Based on 2 QSAR models described in an ECHA dossier, the NOAEL of Copper Gluconate for oral reproductive toxicity in rats was predicted to be 318 mg/kg bw/d and the NOAEL of Copper Gluconate for oral developmental toxicity in rats was predicted to be 793 mg/kg bw/d.

Copper Gluconate was not genotoxic when tested at up to 1 mg/plate in *S. typhimurium* TA97 and TA102 strains, with or without metabolic activation, in an Ames test. Additionally, Copper Gluconate was not mutagenic when evaluated in various in vitro tests using *S. typhimurium* strains TA1535, TA1537, TA1538, and *S. cerevisiae* strain D4, with or without metabolic activation. In a QSAR Toolbox 3.4.0.17 prediction described in an ECHA dossier, Copper Gluconate was predicted to be non-genotoxic in an Ames test (with and without metabolic activation) and in a chromosome aberration test. Additionally, based on the expert rule-based system, Derek Nexus 6.3.0, Copper Gluconate is not predicted to be mutagenic.

After an injection with DEN, male Fischer 344 rats (9 – 12 /group) received 0, 0.001, 0.03, or 0.6% Copper Gluconate in a basal diet for 6 wk in a medium-term liver carcinogenicity bioassay. Numbers of GST-P-positive lesions, single GST-P-positive hepatocytes, 8-oxoguanine-positive hepatocytes, and levels of cell proliferation and apoptosis in the liver were significantly increased in the 0.6% Copper Gluconate group, with and without nitrosamine pre-treatment. The hepatic mRNA expression of *Mt1a, Gadd45α*, *p21*, *TNF- α, IL -1α, Nos2*, and *c-fos* were significantly increased in the 0.6% group, irrespective of nitrosamine treatment, while *p53* expression was significantly increased in the 0.03% and 0.6% groups which received the nitrosamine injection and in the 0.6% group which did not receive the nitrosamine injection. While treatment with Copper Gluconate may have been associated with carcinogenic risk toward the liver at the 0.6% dose, the researchers noted a considerably large safety margin for Copper Gluconate at the human relevant dose of 0.001 and 0.03% (0.001% nearly corresponding to the daily human intake, as a food additive).

In a 13-wk medium-term, multi-organ carcinogenesis assay, male Brl:Han Wistar rats (3/group) were fed a diet containing 0, 0.1, 0.3, 0.48, 0.6, or 1.2% Copper Gluconate, while being exposed to multiple carcinogens (DEN, MNU, DMH, and DHPN). Black stool was found in rats exposed to ≥ 0.3% Copper Gluconate, copper levels in the serum, urine, and liver were significantly increased in rats dosed with 0.6% Copper Gluconate, and marked diffuse granulomas and hepatocellular necrosis were observed in the liver of the single (1) rat in the 1.2% Copper Gluconate group. Copper Gluconate did not exert significant systemic toxicity; however, it was noted that Copper Gluconate may cause toxic and carcinogenic risks to the liver at high doses.

In a 90-d oral toxicity study, evaluating the effects of Copper Gluconate on renal function, a statistically significant increase in renal urea, creatine, sodium, and potassium levels was observed in male albino Swiss rats (8/group) that were administered 3.75, 7.5, or 15 mg/kg Copper Gluconate, in saline, via gavage. These results were indicative of renal failure and the test article was considered nephrotoxic.

A leave-on baby product formulation and a rinse-off adult formulation, each containing 0.00008% Copper Gluconate (0.00004 mg/cm^2) and a rinse-off baby product formulation containing 0.2% Copper Gluconate (0.1 mg/cm^2) were not irritating or sensitizing when tested neat in 3 separate HRIPTs using 210, 211, and 217 subjects, respectively. A powder formulation containing 0.1% Copper Gluconate (up to 0.038 mg/cm^2) was not irritating or sensitizing when tested in distilled water in an HRIPT using 52 subjects. Based on a QSAR model described in an ECHA dossier, the PDII of Copper Gluconate was predicted to be 2.26 in rabbit skin. In another QSAR-based prediction described in an ECHA dossier, Copper Gluconate was predicted to produce an EC3 value of 5.08% in an in vivo LLNA of mice; the test article was predicted to have low to moderate skin-sensitizing potential.

The ocular irritation potential of a face cream containing 0.0025% Copper Gluconate was evaluated in an MTT assay using an in vitro tissue model. The test article was classified as minimally or not irritating to the eyes. Based on a QSAR model for ocular irritation, the MMAS for Copper Gluconate in rabbit eyes was predicted, as described in an ECHA dossier, to be 49.5 out of a maximum damage value of 110; the test article was considered to be a potential mild irritant to the eyes.

In a 12-wk, double-blind, randomized clinical trial, subjects received either a 5 mg capsule of Copper Gluconate or placebo, twice a day. No significant changes in copper, zinc, and magnesium levels were observed in the serum, urine, or hair. Similarly, no significant changes in hematocrit, mean corpuscular volume, serum cholesterol, triglyceride, glutamic-oxaloacetic transaminase, alkaline phosphatase, gamma-glutamyl transferase, or lactate dehydrogenase levels were observed in treated subjects. Serum potassium levels did change from a mean of 4.3 mEq/l to 4 mEq/l ($p < 0.05$). The incidence of nausea, diarrhea, and heartburn was the same in both treated subjects and controls.

Using the in silico tool, VEERMEER Cosmolife, daily exposures to copper from Copper Gluconate were estimated to not exceed 151.2 µg/d in mouthwash, 70 µg/d in make-up removers, and 87.6 µg/d in body lotions. These exposure levels are substantially lower than the RDA values for copper in adults and babies (900 and 340 µg/d), as well as corresponding TUL values (10,000 µg/d and 1000 µg/d).

DRAFT DISCUSSION

[Note: This Discussion is in the draft form, and changes will be made following the Panel meeting.]

This assessment reviews the safety of Copper Gluconate as used in cosmetic formulations. The Panel concluded [TBD].

The Panel considered the lack of systemic toxicity and the GRAS status of Copper Gluconate as a direct human food ingredient, including uses as a dietary supplement and in infant formula. Negative results in several HRIPTs performed using cosmetic formulations containing Copper Gluconate reassured the Panel of dermal safety. Data received from suppliers on the purity of Copper Gluconate as used in cosmetics demonstrated the use of good manufacturing practices to the Panel…[TBD]

The Panel also discussed the issue of incidental inhalation resulting from exposure to this ingredient; for example, Copper Gluconate is reported to be used in face powder formulations (concentration not provided) and could possibly be inhaled. Inhalation toxicity data were not available. Coupled with the small actual exposure in the breathing zone and the low concentrations at which this ingredient is used (or is expected to be used) in potentially inhaled products, the available information indicates that the incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be assessed by the Panel. Therefore, the Panel has found the data insufficient to support the safe use of this cosmetic ingredient applied via an airbrush delivery system.

CONCLUSION

To be determined.

TABLES

Table 1. Chemical properties

Property	Value	Reference
Physical Form	solid; crystalline powder powder fine powder	4,5 6 21CFR184.1260
Color	light blue to bluish-green green	4,5 21CFR184. 1260 6
Odor	odorless	4,5
Formula Weight (g/mol)	453.9 (compared to 63.55 g/mol atomic weight of copper)	7,8
Topological Polar Surface Area (Å2)	283	5
Density (g/ml @ 20 ºC)	1.78	4
Vapor pressure (mmHg @ 20 ºC)	0.01	4
Melting Point (ºC)	155 - 157	4,5
Water Solubility (g/l @ 25 °C)	300	4,5
Solubility		4,5
Soluble	water, alcohol (slightly)	
Insoluble	acetone, ether, organic solvents	
log K_{ow}	-2.98 (estimated)	4

Table 2. Frequency (2023)[13] and concentration (2022)[14] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)
Totals*	**170**	**0.000025 - 0.36**
summarized by likely duration and exposure**		
Duration of Use		
Leave-On	*140*	*0.00008 - 0.008*
Rinse-Off	*30*	*0.000025 - 0.36*
Diluted for (Bath) Use	*NR*	*NR*
Exposure Type*		
Eye Area	13	0.0005 - 0.006
Incidental Ingestion	6	0.36
Incidental Inhalation-Spray	53[a]; 46[b]	0.0008[b]
Incidental Inhalation-Powder	5; 46[b]	0.0008[b]; 0.0008 - 0.003[c]
Dermal Contact	156	0.0008 - 0.1
Deodorant (underarm)	NR	NR
Hair - Non-Coloring	8	0.000025 - 0.0008
Hair-Coloring	NR	NR
Nail	NR	NR
Mucous Membrane	8	0.36
Baby Products	2	0.00008
as reported by product category		
Baby Products		
Baby Shampoos	2	0.00008
Baby Lotions/Oils/Powders/Creams	NR	0.00008
Eye Makeup Preparations		
Eyeliner	NR	0.006
Eye Lotion	7	0.0005
Eye Makeup Remover	1	0.0008
Other Eye Makeup Preparations	5	NR
Hair Preparations (non-coloring)		
Hair Conditioner	NR	0.000025
Rinses (non-coloring)	NR	0.0008
Shampoos (non-coloring)	4	0.000025
Tonics, Dressings, and Other Hair Grooming Aids	1	NR
Other Hair Preparations	1	NR
Makeup Preparations		
Blushers (all types)	2	NR
Face Powders	5	NR
Foundations	5	NR
Lipstick	2	NR
Makeup Bases	1	NR
Makeup Fixatives	3	NR
Other Makeup Preparations	4	0.0025
Oral Hygiene Products		
Mouthwashes and Breath Fresheners	4	NR
Other Oral Hygiene Products	NR	0.36
Personal Cleanliness Products		
Bath Soaps and Detergents	1	NR
Other Personal Cleanliness Products	1	NR
Skin Care Preparations		
Cleansing	17	0.0016 - 0.1
Face and Neck (exc shave)	39	not spray: 0.0008 - 0.003
Body and Hand (exc shave)	7	not spray: 0.0008
Moisturizing	35	not spray: 0.0025
Night	5	not spray: 0.005 – 0.008
Paste Masks (mud packs)	NR	0.0001 - 0.005
Skin Fresheners	7	NR
Other Skin Care Preparations	10	0.0005

NR – not reported

* Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

**likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a 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.

Table 3. Acute toxicity studies

Test Article	Vehicle	Animals/Group	Concentration/Dose	Protocol	LD_{50}/Results	Reference
			DERMAL			
Copper Gluconate	NA	NA	NA	Results from a QSAR model (described in an ECHA dossier); based upon REACH Guidance QSAR R6; was used to predict the acute dermal LD_{50} in rats.	LD_{50} = 2130 mg/kg bw	4
			ORAL			
Copper Gluconate	deionized water	Male Wistar rats (4 – 6/group)	79.5, 156, or 312 mg/kg	Administered via gavage; body weights were recorded for 7 d. Animals were killed on day 7 and samples from the blood and liver were obtained for analysis. Controls received equivalent doses of calcium gluconate.	No animals from the 79.5 and 156 mg/kg groups died. No significant differences in weight gain or hepatic activity (measured via GGT and ALT levels) were observed compared to the control group. Survival rate for the 312 mg/kg group was 31%.	22
Copper Gluconate	water	Wistar rats (5/sex/group)	0, 1800, 2400, or 3200 mg/kg bw	OECD TG 401; administered via gavage; animals were observed for up to 14 d.	LD_{50} = 1709 mg/kg bw (combined for males and females) Deaths/group: -1800 mg/kg: 5 within 48 h -2400 mg/kg: all 10 within 48 h -3200 mg/kg: all 10 within 24 h In the animals that were found dead, local hemorrhages and necrosis were found in the fundus of the stomach, and the intestinal tracts were congested; surviving animals did not exhibit any treatment-related gross abnormalities upon necropsy.	4

ALT – alanine aminotransferase; GGT – gamma-glutamyl transpeptidase; OECD – Organisation for Economic Co-operation and Development; REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals; TG – test guideline

Table 4. Repeated dose toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
				ORAL			
Copper Gluconate	feed	Male Fischer 344 rats (5/group)	2 wk	0, 0.001, 0.03, or 0.6% (0, 10, 300, or 6000 ppm)	The liver was removed and weighed upon study termination. Liver tissue was fixed for histopathological analysis and the remainder was assessed for genes related to metal metabolism (*Mt1a*), apoptosis (*Gadd45α*, *p21*, *p53*), inflammation (*TNF*-α, *IL-1*α, *Nos2*), and normal cell growth (*c-fos*).	The test article did not affect final body weight, liver weight, or food consumption and no gross or histological changes were observed in the liver of treated animals, compared to controls. Hepatic mRNA expression of metal metabolism-related gene *Mt1a* and apoptosis-related gene *Gadd45α* were significantly increased in the 0.6% group. The expression of apoptosis-related gene *p21* was significantly increased in the 0.03 and 0.6% groups. The expression of *p53* (apoptosis-related), *TNF*- α, *IL-1*α, *Nos2* (inflammation-related), and *c-fos* (related to cell growth) expression were not affected at any dose level.	23
Copper Gluconate	feed	Male and female rats (number not specified)	6 mo (24 wk)	0.006 or 0.06% in the diet (mean consumption of 3.46 or 34.9 mg/kg/d)	No further details were provided.	No adverse effects were noted in food consumption, body weight gain, urinalysis, or gross and microscopic examination of tissues and organs at necropsy. Copper content was elevated in the kidneys of test animals fed the diet containing 0.06% Copper Gluconate.	24
Copper Gluconate	feed	Rats (25/sex/group)	Up to 44 wk	1.14% in the diet (equivalent to 0.16% copper)	A control group was also maintained. No further details were provided.	Significant growth retardation was discernible at 26 wk, compared to controls. Over 80% of the animals died between week 17 and week 35. Hematology and urine components were within the normal range except for high blood non-protein nitrogen in males. Upon necropsy, hypertrophied uteri, ovaries, seminal vesicles and hypertrophied stomachs, occasional ulcers, bloody mucus in the intestinal tract, and bronzed kidneys and livers were observed. Abnormal hepatic and renal changes, varying degrees of testicular damage, and a marked depression in tissue storage of iron was also observed. Chronic exposure to 1.14% Copper Gluconate in the diet was considered toxic.	3,25
Copper Gluconate	feed	Male and female Beagle dogs (6/group/sex)	Up to 1 yr (52 wk)	0.012, 0.06, or 0.24% in the diet (equivalent to 3, 15, or 60 mg/kg/d)	Clinical chemistry parameters and urine samples were obtained at 4, 13, or 26 wk. Interim sacrifice and necropsy of 2 animals/sex/group was performed after 6 mo of treatment . No further details were provided.	After 6 mo of dosing, no differences were noted in overall health, hematology, urinalysis, food consumption, or body weight gain, between test animals and controls. After 1 yr of dosing, 1 out of 12 dogs from the 0.24% group exhibited minimal liver function, which was reversible with a 12-wk withdrawal period. No test-article related deaths occurred and gross or microscopic pathologic lesions were not observed upon sacrifice. Accumulation of copper was seen in the liver, kidneys, and spleen in the 0.24% group; no other test article-related effects were observed at the lowest dose or in any dog.	3,25
Copper Gluconate	drinking water	Male C57BL/6J mice (number not specified)	animal lifetime	1st experiment: 0.005 M Copper Gluconate (317 ppm copper) in ~ 4 ml water/d), ad libitum 2nd experiment: 0.0005 or 0.001 M Copper Gluconate (12.7 or 63.5 ppm copper), ad libitum	Mice also received copper in the diet (incidentally containing 18 ppm copper in the ash) from the beginning of the study; controls received distilled water, ad libitum 1st experiment: mice received Copper Gluconate in drinking water from 58 d of age. 2nd experiment: mice received Copper Gluconate in drinking water from 31 d of age.	Survival curves and lifespan were significantly reduced by 11.8% (0.0005 M; $p > 0.05$) and 14.7% (0.001 M; $p < 0.01$) for mice in the 2nd experiment and by14.4% (0.005 M; $p < 0.01$) for treated mice in the 1st experiment. These results indicated the absence of dose-response relationship for survival. Animals that consumed Copper Gluconate weighed slightly less than controls throughout the experiment and died earlier.	21

Table 4. Repeated dose toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
Copper Gluconate	In food (fruits or sauces)	Adult tufted Capuchin monkeys - treated group (2/sex) - age-matched controls (3 males/1 female)	3 yr (156 wk)	5 mg/d, increased to 7.5 mg/d (of copper as Copper Gluconate) over initial 2 mo	The monkeys were 3 - 3.5 yr old at enrollment. Blood samples were collected every 2nd month during the 1st year and every 3rd month thereafter. Hematological indicators, liver aminotransferases (serum aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyl transpeptidase), and serum and hair copper concentrations were measured. The liver was biopsied every 3rd month during the 1st year and every 6 mo thereafter, to assess general hepatic structure and visualize copper distribution. At the end of the experiment, liver biopsies were assessed for the relative abundance of 4 transcripts encoding proteins related to copper uptake, storage and metabolism (*MT2a*, *APP*, *DMT1*, and *CTR1*) and 3 proteins related to hepatic responses to injury (*HGF*, *TGFβ*, and *NFκB*).	No differences in food intake or bodyweight were observed between the treated animals and controls. Hemoglobin and mean corpuscular volume were significantly lower and free erythrocyte protoporphyrin was significantly greater in treated animals compared to controls; liver aminotransferases did not differ between groups. At 24 mo, levels of Ki67 and MT1 proteins in liver tissue were significantly greater in treated animals compared to controls. When assessed after 36 mo, the hepatic mRNA expression of *NFκB*, *HGF*, and *TGFβ* was significantly greater in the treated animals, compared to controls, with no further evidence of clinical, hematological, or histological evidence of liver damage. Copper hair and liver concentrations were significantly greater (4 - 5 times that of controls) in treated animals.	26
Copper Gluconate	Cow milk infant formula	Young Capuchin monkeys (2/sex) -treated group -age-matched controls	3 yr (156 wk)	3.5 mg/d, increased to 5.5 mg/d (of copper, as Copper Gluconate) over initial 2 mo	The monkeys were newborn at enrollment, and received a daily Copper Gluconate dose in milk formula, adjusted to the monkey's body weight every 2 wk, even after fruits and vegetables were introduced to the diet at 4 - 6 mo. Blood, hair and liver samples were collected and analyzed as described above (analyses of proteins related to hepatic injury were not performed).	No differences in food intake or body weight were observed, including weight gain by age or time of exposure, between the treated animals and controls. Gamma glutamyl-transpeptidase was significantly greater in treated animals compared to controls; no differences were observed in the other hematological indicators or liver aminotransferases. At 24 mo, levels of the antibodies Ki67 and MT1 in liver tissue were greater in treated animals compared to controls. After 36 mo, copper hair and liver concentrations were significantly greater in treated animals (4 -5 times that of controls).	26
COMPUTATIONAL							
Copper Gluconate	NA	NA	NA	NA	Results from a QSAR model (described in an ECHA dossier); based on REACH guidance QSARs R.6 and were used to predict the oral LOAEL for Copper Gluconate in rats.	LOAEL = 94.7 mg/kg bw/d; According to this value and the GHS/CLP classification, the STOT RE -2 designation, indicating presumed toxicity to specific organs with repeated exposure, was considered applicable.	4

APP – amyloid precursor protein; *c-fos* – protein c-Fos; CLP - Classification, Labelling, and Packaging regulation; *CTR1* – copper transporter 1; *DMT1* – divalent metal transporter 1; DMSO - dimethyl sulfoxide; *Gadd45α* - growth arrest and DNA damage inducible alpha; GHS - Globally Harmonized System; *HGF* – hepatocyte growth factor; *IL-1α* - interleukin 1-alpha; Ki67 – protein biomarker for cell proliferation; LOAEL – lowest-observed-adverse-effect-level; MT1 – metallothionein 1; *MT1a* – metallothionein 1a; *MT2a* – metallothionein 2a; mRNA – messenger ribonucleic acid; NA – not applicable; *NFκβ* - nuclear factor kappa-light-chain-enhancer of activated B cells; *Nos2* – nitric oxide synthase 2; p53 – tumor protein p53; QSAR – quantitative-structure activity relationship; REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals; STOT RE- specific target organ toxicity, repeated exposure; *TGFβ* - transforming growth factor-β; *TNF*-α - tumor necrosis factor alpha

Table 5. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
ORAL						
Copper Gluconate	Not specified	Male albino rats (8/group)	3.75, 7.5, or 15 mg/kg/d	Animals were dosed via gavage for 90 d. Two control groups received either 1 ml of saline or 0.5 ml DMSO for the duration of the study. Several antioxidant enzymes activities in the testis tissue of rats were determined spectrometrically.	Copper Gluconate dosing did not significantly affect catalase levels but did significantly reduce glutathione and superoxide dismutase levels (at the medium and high dose), while increasing malondialdehyde levels, compared to controls. These findings are indicative of the development of oxidative stress.	27
Copper Gluconate	Not specified	Female Swiss-Webster mice (20/group)	0, 0.1, 3, 30 mg/kg/d	The test article was administered, via gavage, to pregnant mice on days 6 to 14 of gestation.	Neither embryotoxic nor teratogenic. The average length and weight of the fetuses, their number per litter and the incidence of skeletal and soft tissue abnormalities did not differ from control animals.	3,25
Copper Gluconate	Not specified	Female albino Wistar rats (number not specified)	0, 0.1, 3, 30 mg/kg/d	The test article was administered, via gavage, to pregnant rats on days 5 to 15 of gestation.	Neither embryotoxic nor teratogenic. Weekly body weights and food intake were similar among all groups. Corpora lutea, implantation sites, implantation loss were not affected by treatment. The mean number of fetuses/litter, fetal viability, and resorption sites in the treated groups did not differ from the control group. Measurements of fetal weight and length as well as incidence of skeletal and soft tissue abnormalities were also unaffected by treatment.	3,25
Copper Gluconate	Not specified	Male and female Wistar rats (males: 10/group; females: 20/group)	Female rats: 0, 3, or 30 mg/kg/d Male rats: 0 or 3 mg/kg/d	Female rats were dosed (via gavage) with Copper Gluconate 15 d prior to mating with untreated males, during gestation, and for 21 d postpartum. Two groups of male rats were treated 60 d prior to mating (via gavage). One group of treated males was mated with untreated females and the 2nd group of treated males was mated with females who had also received 3 mg/kg/d Copper Gluconate 60 d prior to mating. A third group of untreated males mated with untreated females served as controls.	Male rat reproductive performance was not affected by Copper Gluconate administration. No significant differences were observed between the percentage of pregnancies, the number and distribution of embryos in each uterine horn, implantation sites, resorption sites, duration of gestation, mean number of fetuses and live pups per litter, litter size, stillborn and live born numbers, gross anomalies and mean weight per pup, compared to controls. At the end of the 21-d postpartum period, necropsies of the dams and pups from all groups revealed a lack of visceral abnormalities. Under the conditions of this study, the researchers concluded that Copper Gluconate did not affect the reproductive performance of either male or female rats.	3,25
COMPUTATIONAL						
Copper Gluconate	NA	NA	NA	As described in an ECHA dossier, an oral NOAEL reproductive toxicity in rats was determined using a QSAR model following the REACH Guidance on QSARs and Grouping of Chemicals R.6. However, the specifics of how these values were derived were not provided.	NOAEL = 318 mg/kg bw/d	4
Copper Gluconate	NA	NA	NA	as above, but for developmental toxicity in rats	NOAEL = 793 mg/kg bw/d	4

DMSO – dimethyl sulfoxide; NA – not applicable; NOAEL – no-observed-adverse-effect-level; QSAR - quantitative-structure activity relationship; REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals

Table 6. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
SENSITIZATION						
HUMAN						
Leave on baby product containing 0.00008% Copper Gluconate	applied neat	0.2 ml/mg Copper Gluconate dose applied: 0.00004 mg/cm^2	210 subjects	HRIPT; occlusive conditions (patch size 4 cm^2); 9 induction patches; challenge patch was applied to an untreated site after 2 wk. Challenge readings were taken 24, 48, 72, and 96 h after patch removal.	non-irritating; non-sensitizing	30
Rinse-off adult product containing 0.00008% Copper Gluconate	applied neat	0.2 ml/mg Copper Gluconate dose applied: 0.00004 mg/cm^2	211 subjects	HRIPT; occlusive conditions (patch size 4 cm^2); 9 induction patches; challenge patch was applied to an untreated site after 2 wk. Challenge readings were taken 24, 48, 72, and 96 h after patch removal.	non-irritating; non-sensitizing	31
Powder containing 0.1% Copper Gluconate	distilled water	0.1 – 0.15 g Copper Gluconate dose applied: 0.025 – 0.038 mg/cm^2 (equivalent to 0.0036 – 0.0054 mg/cm^2 copper)	52 subjects	HRIPT; occlusive conditions; 9 induction patches (~0.025 – 0.038 mg/cm^2 of test material per patch); challenge patch was applied to an untreated site after ~ 2 wk. Challenge readings were taken 24 and 72 h after patch removal.	non-irritating; non-sensitizing	32
Rinse-off baby product containing 0.2% Copper Gluconate	applied neat	0.2 ml/mg Copper Gluconate dose applied: 0.1 mg/cm^2	217 subjects	HRIPT; occlusive conditions (patch size 4 cm^2); 9 induction patches; challenge patch was applied to an untreated site after 2 wk. Challenge readings were taken 24, 48, 72, and 96 h after patch removal.	non-irritating; non-sensitizing	33
COMPUTATIONAL						
Copper Gluconate	NA	NA	NA	Results from a QSAR model (described in an ECHA dossier) are based on REACH guidance and were used to predict a PDII of 2.26 in rabbit skin.	Prediction of being non-irritating and non-sensitizing	4
Copper Gluconate	NA	NA	NA	Results from a QSAR model (described in an ECHA dossier) were used to predict that the EC3 for Copper Gluconate in a local lymph node proliferative assay is 5.08%.	Based on an EC3 value > 2%, Copper Gluconate was classified as having low to moderate skin-sensitizing potential (Skin Sensitizer Category 1B, under GHS category 1)*	4

ECHA – European Chemicals Agency; GHS – Globally Harmonized System; EC3 – effective concentration needed to induce a 3-fold increase in a positive reaction in a local lymph node assay; HRIPT – human repeated insult patch test; NA – not applicable; PDII – primary dermal irritation index; QSAR – quantitative structure-activity relationship; REACH – Registration, Evaluation, Authorisation, and Restriction of Chemicals
*substances that show a low to moderate frequency of occurrence in humans and/or low to moderate potency in animals and can be presumed to potentially produce significant sensitization in humans.

Table 7. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
IN VITRO						
Face cream containing 0.0025% Copper Gluconate	applied neat	100 µl	EpiOcular™ tissues, tested in duplicate	Tissues were treated for 4, 8, 16 and 24 h in a MTT assay. 0.3% Triton X-100 and distilled water served as positive and negative controls, respectively. Because the treatment with the test article reduced MTT in the absence of viable tissue, a killed control experiment was conducted. Little or no direct MTT reduction was observed in test article-treated killed controls compared to negative controls with killed cells; MTT reduction in the test article-treated viable tissue was ascribed to the viable cells.	ET_{50} > 24 h (compared to 24 min for the positive control); classified as minimally or not irritating Cell viability after each treatment time: After 4 h: 111.1% 8 h: 107.2% 16 h: 92.2% 24 h: 63.7%	35
COMPUTATIONAL						
Copper Gluconate	NA	NA	NA	Using QSAR Toolbox 3.4.0.17 and REACH guideline on QSAR, the MMAS for Copper Gluconate was predicted in rabbit eyes.	MMAS = 49.5 Copper Gluconate predicted to be mildly toxic, considering the maximum value for damage to the cornea, conjunctiva, and iris is 110. Based on GHS criteria, Copper Gluconate was classified as a possibly mild irritant to the eyes (Category 2B).	4

ET_{50} – time taken in minutes for the test article to reduce the viability of the skin model to 50%, GHS – globally harmonized system; compared to control cultures ; MMAS – modified maximum average score; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

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Concentration of Use by FDA Product Category – Copper Gluconate

Product Category	Maximum Concentration of Use
Baby shampoos	0.00008%
Baby lotions, oils, and creams	0.00008%
Eyeliners	0.006%
Eye lotions	0.0005%
Eye makeup removers	0.0008%
Hair conditioners	0.000025%
Rinses (noncoloring)	0.0008%
Shampoos (noncoloring)	0.000025%
Other makeup preparations	0.0025%
Other oral hygiene products	0.36%
Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	0.0016-0.1%
Face and neck products Not spray	 0.0008-0.003%
Body and hand products Not spray	 0.0008%
Moisturizing products Not spray	 0.0025%
Night products Not spray	 0.005-0.008%
Paste masks and mud packs	0.0001-0.005%
Other skin care preparations	0.0005%

Information collected in 2022

Table prepared: July 6, 2022

Revised April 12, 2024: Baby shampoo containing 0.2% was never marketed; add baby lotions oils and creams (remove other baby products); add eye makeup removers; add hair rinses (noncoloring); add other oral hygiene products; skin cleansing lowest value changed from 0.0023 to 0.0016%; face and neck products lowest value changed from 0.0005 to 0.0008%; added body and hand products; moisturizing products lowest concentration (0.0005%) removed; night products added 0.008%; other suntan preparations no longer reported

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 13, 2024

SUBJECT: Copper Gluconate

Anonymous. 2021. Product sheet (Copper Gluconate, USP Powder).

Anonymous. 2024. Certificate of analysis (Copper Gluconate, USP Powder).

Anonymous. 2024. Elemental impurity profile (Copper Gluconate, USP Powder).

PRODUCT SHEET

Product Name: Copper Gluconate, USP Powder
Product Code: 2290
Chemical Formula: $Cu(C_6H_{11}O_7)_2$
Mol. Weight: 453.9
CAS Number: 527-09-3
Use: Nutrient; Dietary Ingredient
Description: Blue-green powder

SPECIFICATIONS

ID Test A	Pass	Sulfate	0.05% Maximum
ID Test B	Pass	Mesh Thru 80	95% Minimum
Appearance	Blue-Green Powder	Arsenic	3 ppm Maximum
Assay	98.0% - 102.0%	Lead	5 ppm Maximum
Copper Content	Report	Aerobic Plate Count	10^3 cfu/g Maximum
Reducing Substances	1.0% Maximum	Yeast and Mold Count	10^2 cfu/g Maximum
Chloride	0.07% Maximum	Total Coliforms	Negative

Mineral Content: Cu (as-is): 13.7% - 14.3%
(For formulation guidance/ Not a specification)

Containers: 55lb/25kg fiber drums; 55lb/25kg large carton

Storage: Store in a clean, dry warehouse in the original unopened containers.

Revision Date: 7/14/2021 12:00:00 AM

Copper gluconate is hygroscopic. Product stored in open containers or containers, which are opened repeatedly, will allow moisture absorption to discolor this product. To avoid discoloration, completely use the entire container once opened and obtain samples in a reduced humidity environment and quickly reseal the sampled container.

Certificate of Analysis

Product Name:	Copper Gluconate, USP Powder	**Product Code:**	2290
Date:	May 13, 2024	**Retest Date:**	February 27, 2023
Manufacture Location:		**Country of Origin:**	USA
Lot Number:			
Date of Manufacture:	February 27, 2020		

TEST	SPECIFICATION	ANALYSIS
ID Test A:	Pass	Pass
ID Test B:	Pass	Pass
Appearance:	Blue-Green Powder	Pass
Assay:	98.0% - 102.0%	100.2 %
Copper Content:	Report	14.0 %
Reducing Substances:	1.0% Maximum	0.21 %
Chloride:	0.07% Maximum	< 0.07%
Sulfate:	0.05% Maximum	< 0.05%
Mesh Thru 80:	95% Minimum	100.0 %
Arsenic:	3 ppm Maximum	0.10 ppm
Lead:	5 ppm Maximum	0.02 ppm
Aerobic Plate Count:	10^3 cfu/g Maximum	< 1000 cfu/g
Yeast and Mold Count:	10^2 cfu/g Maximum	< 100 cfu/g
Total Coliforms:	Negative	Negative

Elemental Impurity Profile

Product name & Product code:	Copper Gluconate, USP, Powder,		
ELEMENTAL IMPURITY	**Present Above Threshold Value?***	**Typical Level**	**Threshold Value (ppm)**
Cd – Cadmium	No	Less than or equal to threshold value	0.5
Pb – Lead	See CoA specifications	See CoA specifications	0.5
As – Arsenic	See CoA specifications	See CoA specifications	1.5
Hg – Mercury	No	Less than or equal to threshold value	3.0
Co – Cobalt	No	Less than or equal to threshold value	5.0
V – Vanadium	No	Less than or equal to threshold value	10.0
Ni – Nickel	No	Less than or equal to threshold value	20.0
Tl – Thallium	No	Less than or equal to threshold value	0.8
Au – Gold	No	Less than or equal to threshold value	30.0
Pd – Palladium	No	Less than or equal to threshold value	10.0
Ir – Iridium	No	Less than or equal to threshold value	10.0
Os – Osmium	No	Less than or equal to threshold value	10.0
Rh – Rhodium	No	Less than or equal to threshold value	10.0
Ru – Ruthenium	No	Less than or equal to threshold value	10.0
Se – Selenium	No	Less than or equal to threshold value	15.0
Ag – Silver	No	Less than or equal to threshold value	15.0
Pt – Platinum	No	Less than or equal to threshold value	10.0
Li – Lithium	No	Less than or equal to threshold value	55.0
Sb – Antimony	No	Less than or equal to threshold value	120
Ba – Barium	No	Less than or equal to threshold value	140
Mo – Molybdenum	No	Less than or equal to threshold value	300
Cu – Copper	No	Less than or equal to threshold value	300
Sn – Tin	No	Less than or equal to threshold value	600
Cr – Chromium	No	Less than or equal to threshold value	1100

***Threshold Value** – In accordance with ICH-Q3D *Elemental Impurities* and USP 232 requirements. Elements above threshold value are monitored and reported upon request. Threshold values are not specifications.

Title:

Date: April 5, 2024

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: April 10, 2024

SUBJECT: Copper Gluconate

Anonymous. 2011. Clinical safety evaluation: Repeated insult patch test (powder containing 0.1% Copper Gluconate).

The report (p.3) states: that "approximately 25-28 mg/cm^2 of the test material" (Parke-Davis Readi-Bandage® occlusive patch) was placed on the backs of the test subjects.

The test material contained 0.1% Copper Gluconate. Therefore, the subjects were exposed to approximately 25-38 µg/cm^2 Copper Gluconate. Multiplying this by the ratio of the molecular weight of copper compared to Copper Gluconate (64/454), this is approximately 3.6-5.4 µg/cm^2 copper.

[redacted]

FINAL REPORT

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

Powder product contains 0.1% Copper Gluconate

Sponsor

[redacted]

Sponsor Representative

[redacted]

Clinical Testing Facility

[redacted]

[redacted]

Date of Final Report

2-14-11

[redacted]

[redacted]

SIGNATURE PAGE

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

QUALITY ASSURANCE STATEMENT

This study [redacted] was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in 21 CFR Part 50 (Protection of Human Subjects – Informed Consent) and the Standard Operating Procedures of [redacted]

For purposes of this clinical study:

_X_	Informed Consent was obtained.
____	Informed Consent was not obtained.
_X_	An IRB review was not required.
____	An IRB review was conducted and approval to conduct the proposed clinical research was granted.

To assure compliance with the study protocol, the Quality Assurance Unit completed an audit of the applicable study records and report. This report is considered a true and accurate reflection of the testing methods and source data.

[redacted]

11 Feb 11
Date

[redacted]

[redacted]

TABLE OF CONTENTS

[redacted]

[redacted]

CLINICAL SAFETY EVALUATION

REPEATED INSULT PATCH TEST

[redacted]

1.0 OBJECTIVE

The objective of this study was to determine the irritation and/or sensitization potential of the test article after repeated application under occlusive patch test conditions to the skin of human subjects (non-exclusive panel).

2.0 SPONSOR

[redacted]

2.1 Sponsor Representative

[redacted]

3.0 CLINICAL TESTING FACILITY

The study was conducted by:

[redacted]

4.0 CLINICAL INVESTIGATORS

Study Director:
Principal Investigator:
Medical Investigator:

[redacted]

5.0 STUDY DATES

Study initiation: December 15, 2010

Final evaluation: January 28, 2011

[redacted]

[redacted]

6.0 ETHICS

6.1 Ethical Conduct of the Study

This study was conducted in accordance with the intent and purpose of Good Clinical Practice regulations described in Title 21 of the U.S. Code of Federal Regulations (CFR), the Declaration of Helsinki and/or [redacted] Standard Operating Procedures.

6.2 Subject Information and Consent

This study was conducted in compliance with CFR Title 21, Part 50 (Informed Consent of Human Subjects). Informed Consent was obtained from each subject in the study and documented in writing before participation in the study. A copy of the Informed Consent was provided to each subject.

7.0 TEST MATERIAL

The test article used in this study was provided by:

[redacted]

It was received on December 7, 2010 and identified as follows:

[redacted]

Description
Peach Powder*

*The test article was supplied as a composite by the Sponsor and was prepared with distilled water to form a paste prior to application to the patch.

8.0 TEST SUBJECTS

At least 50 male and female subjects ranging in age from 18 to 79 years were to be empanelled for this test.

The subjects chosen were to be dependable and able to read and understand instructions. The subjects were not to exhibit any physical or dermatologic condition that would have precluded application of the test article or determination of potential effects of the test article.

[redacted]

[redacted]

9.0 TEST PROCEDURE

The 9 Repeated Insult (occlusive) Patch Test (9-RIPT) was conducted as follows:

9.1 Induction Phase

A sufficient amount of the test article (approximately 0.1 g – 0.15 g) was placed onto a Parke-Davis Readi-Bandage® occlusive patch (approximately 25 - 38 mg/cm^2 of test material) and applied to the back of each subject between the scapulae and waist, adjacent to the spinal mid-line. This procedure was performed by a trained technician/examiner and repeated every Monday, Wednesday and Friday until 9 applications of the test article had been made.

The subjects were instructed to remove the patch 24 hours after application. Twenty-four hour rest periods followed the Tuesday and Thursday removals and 48-hour rest periods followed each Saturday removal. Subjects returned to the Testing Facility and the site was scored by a trained examiner just prior to the next patch application.

If a subject developed a positive reaction of a level 2 erythema or greater during the Induction phase or if, at the discretion of the Study Director, the skin response warranted a change in site, the patch was applied to a previously unpatched, adjacent site for the next application. If a level 2 reaction or greater occurred at the new site, no further applications were made. However, any reactive subjects were subsequently Challenge patch tested.

9.2 Challenge Phase

After a rest period of approximately 2 weeks (no applications of the test article), the Challenge patch was applied to a previously unpatched (virgin) test site. The site was scored 24 and 72 hours after application. All subjects were instructed to report any delayed skin reactivity that occurred after the final Challenge patch reading. When warranted, selected test subjects were called back to the Clinic for additional examinations and scoring to determine possible increases or decreases in Challenge patch reactivity.

Dermal responses for both the Induction and Challenge phases of the study were scored according to the following 6-point scale:

0 = No evidence of any effect
+ = 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 (eg, edema, dryness, hypo- or hyperpigmentation) were appropriately recorded on the data sheet and described as mild, moderate or severe.

[redacted]

[redacted]

9.0 TEST PROCEDURE (CONT'D)

9.3 Data Interpretation

Edema, vesicles, papules and/or erythema that persist or increase in intensity either during the Induction and/or Challenge phase may be indicative of allergic contact dermatitis. Allergic responses normally do not resolve or improve markedly at 72-96 hours.

Exceptions to typical skin reactions may occur. These may include, but not be limited to, symptoms of allergic contact sensitivity early in the Induction period to one or more test products. When this occurs in one subject, such a reaction usually suggests either an idiosyncratic response or that the subject had a pre-exposure/sensitization to the test material or component(s) of the test material or a cross-reactivity with a similar product/component. Data for such reactions will be included in the study report but will not be included in the final study analysis/conclusion of sensitization.

10.0 RESULTS AND DISCUSSION

(See Table 2 for Individual Scores)

A total of 55 subjects (17 males and 38 females ranging in age from 19 to 69 years and 18 of whom had sensitive skin) were empanelled for the test procedure. Fifty-two (52/55) subjects satisfactorily completed the test procedure on Test Article: [redacted] Three (3/55) subjects discontinued for personal reasons unrelated to the conduct of the study. Discontinued panelist data are shown up to the point of discontinuation, but are not used in the Conclusions section of this final report.

Induction Phase Summary

Test Article	Induction Scores (Number of Responses)						Evidence of Irritation
	0.5	1	2	3	4	Other	
[redacted]	0	0	0	0	0	0	No

Challenge Phase Summary

Test Article	Challenge Scores (Number of Responses)						Evidence of Sensitization
	0.5	1	2	3	4	Other	
[redacted]	0	0	0	0	0	0	No

There was no skin reactivity observed at any time during the course of the study.

11.0 CONCLUSIONS

Under the conditions of a repeated insult (occlusive) patch test procedure conducted in 52 subjects (33% with sensitive skin), Test Article: [redacted] was "Dermatologist-Tested" and was not associated with skin irritation or allergic contact dermatitis in human subjects.

[redacted]

TABLE 1

SUBJECT DEMOGRAPHICS

Test Article: [redacted]

Subject No.	[redacted]	Age	Sex	Race	Sensitive Skin
1		45	F	CA	Yes
2		48	F	CA	Yes
3		69	M	CA	Yes
4		36	F	HS	Yes
5		64	F	HS	Yes
6		26	F	CA	Yes
7		68	F	HS	Yes
8		38	F	CA	Yes
9		37	F	CA	Yes
10		56	F	CA	No
11		23	F	HS	Yes
12		28	M	HS	Yes
13		63	M	CA	Yes
14		56	F	CA	Yes
15		31	F	HS	Yes
16		29	F	HS	No
17		33	F	HS	No
18		38	F	HS	No
19		67	F	HS	No
20		56	F	CA	No
21		26	F	CA	No
22		65	F	CA	Yes
23		40	F	CA	Yes
24		68	F	CA	No
25		64	M	CA	No
26		49	M	CA	No
27		59	F	CA	No
28		62	F	CA	No

Subject No.	[redacted]	Age	Sex	Race	Sensitive Skin
29		28	F	CA	No
30		22	F	CA	No
31		75	M	HS	No
32		50	M	HS	No
33		49	M	CA	No
34		49	F	CA	Yes
35		19	F	CA	No
36		59	M	CA	No
37		54	F	CA	No
38		67	F	CA	No
39		48	F	CA	No
40		54	F	CA	No
41		60	F	CA	No
42		61	M	CA	No
43		54	F	CA	No
44		63	F	CA	No
45		64	F	CA	No
46		64	M	HS	No
47		25	F	CA	No
48		45	F	HS	No
49		20	M	CA	Yes
50		64	F	CA	No
51		22	M	CA	No
52		51	M	CA	No
53		35	M	CA	No
54		25	M	HS	No
55		19	M	CA	No

CA = Caucasian
HS = Hispanic

Shaded area = Discontinued subject

[redacted]

TABLE 2

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - OCCLUSIVE

Test Article: [redacted]

Subj. No.	Induction Evaluation Number									Challenge Virgin Site	
	1	2	3	4	5	6	7	8	9	24hr	72hr
1	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	Discontinued							
9	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	Discontinued							
18	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	Discontinued						
22	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0
30	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = 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)

[redacted]

TABLE 2 (CONT'D)

INDIVIDUAL SCORES

REPEATED INSULT PATCH TEST - OCCLUSIVE

Test Article: [redacted]

Subj. No.	Induction Evaluation Number 1	2	3	4	5	6	7	8	9	Challenge Virgin Site 24hr	72hr
31	0	0	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0	0	0	0
37	0	0	0	0	0	0	0	0	0	0	0
38	0	0	0	0	0	0	0	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0
41	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0	0	0
53	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0	0

Scale:0 = No evidence of any effect
+ = 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)

[redacted]

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: July 30, 2024

SUBJECT: Copper Gluconate

Anonymous. 2024. Regulatory statements - Copper Gluconate (elements).

Elemental Impurity	Symbol	Class	Likely to be present			Typical value	Observation
			Yes	No	Intentionally added		
Arsenic	As	1	☐	☑	☐	<1 ppm	
Cadmium	Cd	1	☐	☑	☐	<0.1 ppm	
Mercury	Hg	1	☐	☑	☐	<0.1 ppm	
Lead	Pb	1	☐	☑	☐	<2 ppm	
Cobalt	Co	2A	☐	☑	☐	<1 ppm	
Nickel	Ni	2A	☐	☑	☐	<5 ppm	
Vanadium	V	2A	☐	☑	☐	<1 ppm	
Silver	Ag	2B	☐	☑	☐	-	Not tested. Not expected based on manufacture knowledge.
Gold	Au	2B	☐	☑	☐	-	Not tested. Not expected based on manufacture knowledge.
Iridium	Ir	2B	☐	☑	☐	-	Not tested. Not expected based on manufacture knowledge.
Osmium	Os	2B	☐	☑	☐	-	Not tested. Not expected based on manufacture knowledge.
Palladium	Pd	2B	☐	☑	☐	-	Not tested. Not expected based on manufacture knowledge.
Platinum	Pt	2B	☐	☑	☐	-	Not tested. Not expected based on manufacture knowledge.
Selenium	Se	2B	☐	☑	☐	<0.1 ppm	
Ruthenium	Ru	2B	☐	☑	☐	-	Not tested. Not expected based on manufacture knowledge.
Rhodium	Rh	2B	☐	☑	☐	-	Not tested. Not expected based on manufacture knowledge.
Thallium	Tl	2B	☐	☑	☐	<0.1 ppm	
Barium	Ba	3	☐	☑	☐	<2 ppm	
Chromium	Cr	3	☐	☑	☐	<0.1 ppm	
Copper	Cu	3	☐	☑	☐	-	Intentional addition: 14%
Lithium	Li	3	☐	☑	☐	<1 ppm	
Antimony	Sb	3	☐	☑	☐	<2 ppm	
Tin	Sn	3	☐	☑	☐	-	Not tested. Not expected based on manufacture knowledge.
Molybdenum	Mo	3	☐	☑	☐	<1 ppm	

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 14, 2024

SUBJECT: Copper Gluconate

Anonymous. 2012. HRIPT of a leave-on baby product containing 0.00008% Copper Gluconate (0.04 µg/cm^2 Copper Gluconate tested).

Anonymous. 2013. HRIPT of a rinse-off baby product containing 0.2% Copper Gluconate (100 µg/cm^2 Copper Gluconate tested).

Anonymous. 2010. HRIPT of a rinse-off adult product containing 0.00008% Copper Gluconate (0.04 µg/cm^2 Copper Gluconate tested).

Study completed in 2012

Product Number	% Copper Gluconate	Product Type	HRIPT Test Yes/No	Occlusivity	Completed Subjects	Did formula induce an allergic response
1	0.00008	Leave on	YES	OCCLUSIVE	210	NO

Product Number 1

Calculation of Amount of Copper Gluconate mg/cm2	
Concentration of Copper Gluconate in Product in %	0.00008
Amount of Product applied to Skin during HRIPT in ml/mg	0.2
Patch Size cm^2	4
Dose density of product applied to patched skin in mg/cm2	50
Dose Density of Copper Gluconate applied to patch skin in mg/cm2	0.000040

ICDRG Reading scale	
0	No Visible Reaction
±	Faint Minimal Erythema
1	Erythema
2	Intense Erythema, Induration
3	Intense Erythema, Induration, Vesicles
4	Severe reaction with Erythema, Induration, Vesicles (may be weeping)
E	Edema

–	No reading

Details of Test methodolgy and Results	
0	panelist discontinued due to test material reactions
24 hrs	patch duration
9	induction patches
3	weeks induction
2	week rest period
virgin site	challenge
24, 48, 72, 96 hrs post patching	Challenge readings

Grading Scale interpretation	
Low Level Reactions	1
High Level Reaction	2 and above

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

Baby LO 0.00008%
Copper Gluconate

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
1		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
3		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
5		0	0	0	0	0	0	0	0	0	0	0	0	0
6		0	0	0	0	0	0	0	0	0	0	0	0	0
7		0	0	0	0	0	0	0	0	0	0	0	0	0
8		0	0	0	0	0	0	0	0	0	0	0	0	0
9		0	0	0	0	0	0	0	0	0	0	0	0	0
10		0	0	0	0	0	0	0	0	0	0	0	0	0
11		0	0	0	0	0	0	0	0	0	0	0	0	0
12		0	0	0	0	0	0	0	0	0	0	0	0	0
13		0	0	0	0	0	0	0	0	0	0	0	0	0
14		0	0	0	0	0	0	0	0	0	0	0	0	0
15		0	0	0	0	0	0	0	0	0	0	0	0	0
16		0	0	0	0	0	0	0	0	0	0	0	0	0
17		0	0	0	0	0	0	0	0	0	0	0	0	0
18		0	0	0	0	0	0	0	0	0	0	0	0	0
19		0	0	0	0	0	0	0	0	0	0	0	0	0
20		0	0	0	0	0	0	0	0	0	0	0	0	0
21		0	0	0	0	0	0	0	0	0	0	0	0	0
22		0	0	0	0	0	0	0	0	0	0	0	0	0
23		0	0	0	0	0	0	0	0	0	0	0	0	0
24		0	0	0	0	0	0	0	0	0	0	0	0	0
25		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
26		0	0	0	0	0	0	0	0	0	0	0	0	0
27		0	0	0	0	0	0	0	0	0	0	0	0	0
28		0	0	0	0	0	0	0	0	0	0	0	0	0
29		0	0	0	0	0	0	0	0	0	0	0	0	0
30		X	X	X	X	X	X	X	X	X	X	X	X	X
31		0	0	0	X	X	X	X	X	X	X	X	X	X
32		0	0	0	0	0	0	0	0	0	0	0	0	0
33		0	0	0	0	0	0	0	0	0	0	0	0	0
34		0	0	0	0	0	0	0	0	0	0	0	0	0
35		0	0	0	0	0	0	0	0	0	0	0	0	0
36		0	0	0	0	0	0	0	0	0	0	0	0	0
37		0	X	X	X	X	X	X	X	X	X	X	X	X
38		0	0	0	0	0	0	0	0	0	0	0	0	0
39		0	0	0	0	0	0	0	0	0	0	0	0	0
40		0	0	0	0	0	0	0	0	0	0	0	0	0
41		0	0	0	0	0	0	0	0	0	0	0	0	0
42		0	0	0	0	0	0	0	0	0	0	0	0	0
43		0	0	0	0	0	0	0	0	0	0	0	0	0
44		0	0	0	0	0	0	0	0	0	0	0	0	0
45		0	0	0	0	0	0	0	0	0	0	0	0	0
46		0	0	0	0	0	0	0	0	0	0	0	0	0
47		0	0	0	0	0	0	0	0	0	X	X	X	X
48		0	0	0	0	0	0	0	0	0	0	0	0	0
49		0	0	0	0	0	0	0	0	0	0	0	0	0
50		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
51		0	0	0	0	0	0	0	0	0	0	0	0	0
52		0	0	0	0	0	0	0	0	0	0	0	0	0
53		0	0	0	0	0	0	0	0	0	0	0	0	0
54		0	0	0	0	0	0	0	0	0	0	0	0	0
55		0	0	0	0	0	0	0	0	0	0	0	0	0
56		X	X	X	X	X	X	X	X	X	X	X	X	X
57		0	0	0	0	0	0	0	0	0	0	0	0	0
58		0	0	0	0	±	±	0^	0^	0^	0	0	0	0
59		0	0	0	0	0	0	0	0	0	0	0	0	0
60		0	0	0	0	0	0	0	0	0	0	0	0	0
61		0	0	0	0	0	0	0	0	0	0	0	0	0
62		0	0	0	0	0	0	0	0	0	0	0	0	0
63		0	0	0	0	0	0	0	0	0	0	0	0	0
64		0	0	0	0	0	0	0	0	0	0	0	0	0
65		0	0	0	0	0	0	0	0	0	0	0	0	0
66		0	0	0	±	±	0	0	0	0	0	0	0	0
67		0	0	0	0	0	0	0	0	0	0	0	0	0
68		0	0	0	0	0	0	0	0	0	0	0	0	0
69		X	X	X	X	X	X	X	X	X	X	X	X	X
70		0	0	0	0	0	0	0	0	0	X	X	X	X
71		0	0	0	0	0	0	0	0	0	0	0	0	0
72		0	0	0	0	0	0	0	0	0	0	0	0	0
73		0	0	0	0	0	0	0	0	0	0	0	0	0
74		0	0	0	0	0	0	0	0	0	0	0	0	0
75		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
76		0	0	0	0	0	0	0	0	0	0	0	0	0
77		0	0	0	0	0	0	0	0	0	0	0	0	0
78		0	0	0	0	0	0	0	0	0	0	0	0	0
79		0	0	0	0	0	0	0	0	0	0	0	0	0
80		0	0	0	0	0	0	0	0	0	0	0	0	0
81		0	0	0	0	0	0	0	0	0	0	0	0	0
82		0	0	0	0	0	0	0	0	0	0	0	0	0
83		0	0	0	0	0	0	0	0	0	0	0	0	0
84		0	0	0	0	0	0	0	0	0	0	0	0	0
85		0	0	0	0	0	0	0	0	0	0	0	0	0
86		0	0	0	0	0	0	0	0	0	±	0	0	0
87		0	0	0	0	0	0	0	0	0	0	0	0	-
88		0	0	0	0	0	0	0	0	0	0	0	0	0
89		0	0	0	0	0	0	0	0	0	0	0	-	0
90		0	0	0	0	0	0	0	0	0	0	0	0	0
91		0	X	X	X	X	X	X	X	X	X	X	X	X
92		0	0	0	0	0	0	0	0	0	0	0	0	0
93		0	0	0	0	0	0	0	0	0	0	0	0	0
94		0	0	0	0	0	0	0	0	0	0	0	0	0
95		0	0	0	0	0	0	0	0	0	0	0	0	0
96		0	0	0	0	0	0	0	0	0	0	0	0	-
97		0	0	0	0	0	0	0	0	0	0	0	0	0
98		0	0	0	0	0	0	0	0	0	0	0	0	0
99		0	0	0	0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
101		0	0	0	0	0	0	0	0	0	0	-	0	X
102		0	X	X	X	X	X	X	X	X	X	X	X	X
103		0	0	0	0	0	0	0	0	0	0	0	0	0
104		0	0	0	0	0	0	0	0	0	0	0	0	0
105		0	0	0	0	0	0	0	0	0	0	0	0	0
106		0	0	0	0	0	0	0	0	0	0	0	0	0
107		0	0	0	0	0	0	0	0	0	0	0	0	0
108		0	0	0	0	0	0	0	0	0	0	0	0	0
109		0	0	0	0	0	0	0	0	0	0	0	0	0
110		0	0	0	0	0	0	0	0	0	0	0	0	0
111		0	0	0	0	0	0	0	0	0	0	0	0	0
112		0	0	0	0	0	0	0	0	0	0	0	0	0
113		0	0	0	0	0	0	0	0	0	0	-	0	0
114		0	0	0	0	0	0	0	0	0	0	0	0	0
115		±	0	0	0	0	0	0	0	0	0	0	0	0
116		0	0	0	0	0	0	0	0	0	0	0	0	0
117		0	0	0	0	0	0	0	0	0	0	0	0	0
118		0	0	0	0	0	0	0	0	0	0	0	0	0
119		0	0	0	0	0	0	0	0	0	0	0	0	0
120		X	X	X	X	X	X	X	X	X	X	X	X	X
121		0	0	0	1DR	±	±	±^	0	0	0	0	0	0
122		0	0	0	0	0	0	0	0	0	0	0	0	0
123		0	0	0	0	0	0	0	0	0	0	0	0	0
124		0	0	0	0	0	0	0	0	0	0	0	0	0
125		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
126		0	0	0	0	0	0	0	0	0	0	0	0	0
127		0	0	0	0	0	0	X	X	X	X	X	X	X
128		0	0	0	0	0	0	0	0	0	0	0	0	0
129		0	0	0	0	0	0	0	±	±	0	0	0	0
130		0	0	0	0	0	0	0	0	0	0	0	0	0
131		0	0	0	0	0	0	0	0	0	0	0	0	0
132		0	0	0	0	0	0	0	0	0	0	0	0	0
133		0	0	0	0	0	0	0	0	0	0	0	0	0
134		0	0	0	0	0	0	0	0	0	0	0	0	0
135		0	0	0	0	0	0	0	0	0	X	X	X	X
136		0	0	0	0	0	0	0	0	0	0	0	0	0
137		0	0	0	0	0	0	0	0	0	0	0	0	0
138		0	0	0	0	0	0	0	0	0	0	0	0	0
139		0	0	0	0	0	0	0	0	0	0	0	0	0
140		0	0	0	0	0	0	0	0	0	0	0	0	0
141		0	0	0	0	0	0	0	0	0	0	0	0	0
142		0	0	0	0	0	0	0	0	0	0	0	0	0
143		0	0	0	0	0	0	0	0	0	0	0	0	0
144		0	0	0	0	0	0	0	0	0	0	0	0	0
145		0	0	0	0	0	0	0	0	0	0	0	0	0
146		0	0	0	0	0	0	0	0	0	0	0	0	0
147		0	0	0	0	0	0	0	0	0	0	0	0	0
148		0	0	0	0	0	0	0	0	0	0	0	0	0
149		0	0	0	0	0	0	0	0	0	0	-	0	0
150		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
151		0	X	X	X	X	X	X	X	X	X	X	X	X
152		0	0	0	0	0	0	0	0	0	0	0	0	0
153		0	0	0	0	0	0	0	0	0	0	0	0	0
154		0	0	0	0	0	0	0	0	0	0	0	0	0
155		0	0	0	0	0	0	0	0	0	0	0	0	0
156		0	0	0	0	0	0	0	0	0	0	0	0	0
157		0	0	0	0	0	0	0	0	0	0	0	0	0
158		0	0	0	0	0	0	0	0	0	0	0	0	0
159		0	0	0	0	0	0	0	0	0	0	0	0	0
160		X	X	X	X	X	X	X	X	X	X	X	X	X
161		0	X	X	X	X	X	X	X	X	X	X	X	X
162		0	0	0	0	0	0	0	0	0	0	0	0	0
163		0	0	0	0	0	0	0	0	0	0	0	0	0
164		0	0	0	0	0	0	0	0	0	0	0	0	0
165		0	0	0	0	0	0	0	0	0	0	0	0	0
166		0	0	0	0	0	0	0	0	0	0	0	0	0
167		0	0	0	0	0	0	0	0	0	0	0	0	0
168		0	0	0	0	0	0	0	0	0	0	0	0	0
169		0	0	0	0	0	0	0	0	0	0	0	0	0
170		0	0	0	0	0	0	0	0	0	0	0	0	0
171		0	0	0	0	0	0	0	0	0	0	0	0	0
172		0	0	0	0	0	0	0	0	0	0	0	0	0
173		0	0	0	0	0	0	0	0	0	0	0	0	0
174		0	0	0	0	0	0	0	0	0	0	0	0	0
175		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
176		0	X	X	X	X	X	X	X	X	X	X	X	X
177		0	0	0	0	0	0	0	0	0	0	0	0	0
178		0	0	0	0	0	0	0	0	0	0	-	0	0
179		0	0	0	0	0	X	X	X	X	X	X	X	X
180		0	X	X	X	X	X	X	X	X	X	X	X	X
181		0	0	0	0	0	0	0	0	0	0	0	0	0
182		0	0	0	0	0	0	0	0	0	0	0	0	0
183		0	0	0	0	0	0	0	0	0	0	0	0	0
184		0	0	0	0	0	0	0	0	0	0	0	0	0
185		0	0	0	0	0	0	0	0	0	0	0	0	0
186		0	0	0	0	0	0	0	0	0	0	0	0	0
187		0	0	0	0	0	0	0	0	0	0	0	0	0
188		0	0	0	0	0	0	0	0	0	0	0	0	0
189		0	0	0	0	0	0	0	0	0	0	0	0	0
190		0	0	0	0	0	0	0	0	0	0	0	0	0
191		0	0	0	0	0	0	0	0	0	0	-	0	0
192		X	X	X	X	X	X	X	X	X	X	X	X	X
193		X	X	X	X	X	X	X	X	X	X	X	X	X
194		0	0	0	0	0	0	0	0	0	0	0	0	0
195		0	0	0	0	0	0	0	0	0	0	0	0	0
196		0	0	0	0	0	0	0	0	0	0	0	0	0
197		X	X	X	X	X	X	X	X	X	X	X	X	X
198		0	0	0	0	0	0	0	0	0	0	0	0	0
199		0	0	0	0	0	0	0	0	0	0	0	0	0
200		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
201		0	0	0	0	0	0	0	0	0	0	0	0	0
202		0	0	0	0	0	0	0	0	0	0	0	0	0
203		0	0	0	0	0	0	0	0	0	0	0	0	0
204		0	0	0	0	0	0	0	0	0	0	0	0	0
205		0	0	0	0	0	0	0	0	0	0	0	0	0
206		0	0	0	0	0	0	0	0	0	0	0	0	0
207		0	0	0	0	0	0	0	0	0	0	0	0	0
208		X	X	X	X	X	X	X	X	X	X	X	X	X
209		0	X	X	X	X	X	X	X	X	X	X	X	X
210		0	0	0	0	0	0	0	0	0	X	X	X	X
211		0	0	0	0	0	0	0	0	0	0	0	0	0
212		0	0	0	0	0	0	0	0	0	0	0	0	0
213		0	0	0	0	0	0	0	0	0	0	0	0	0
214		0	0	0	0	0	0	0	0	0	0	0	0	0
215		0	0	0	0	0	0	0	0	0	0	0	0	0
216		0	0	0	0	0	0	0	0	0	0	0	0	0
217		0	0	0	0	0	0	0	0	0	0	0	0	0
218		0	0	0	0	0	0	0	0	0	X	X	X	X
219		0	0	0	0	0	0	0	0	0	0	0	0	0
220		0	0	0	0	0	0	0	0	0	0	0	0	0
221		0	0	0	0	0	0	0	0	0	0	0	0	0
222		0	0	0	0	0	0	0	0	0	0	0	0	0
223		0	0	0	0	0	0	0	0	0	0	0	0	0
224		0	X	X	X	X	X	X	X	X	X	X	X	X
225		0	X	X	X	X	X	X	X	X	X	X	X	X

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
226		0	0	0	0	0	0	0	0	0	0	0	0	0
227		0	0	0	0	0	0	0	0	0	0	0	0	0
228		0	0	0	0	0	0	0	0	0	0	0	0	0
229		0	0	0	0	0	0	0	0	0	0	0	0	0
230		0	0	0	0	0	0	0	0	0	0	0	0	0
231		0	0	0	0	0	X	X	X	X	X	X	X	X
232		0	0	0	0	0	0	0	0	0	0	0	0	0
233		0	0	0	0	0	0	0	0	0	0	0	0	0
234		0	0	X	X	X	X	X	X	X	X	X	X	X
235		0	0	0	0	0	0	0	0	0	0	0	0	0
236		0	0	0	0	0	0	0	0	0	0	0	0	0
237		0	0	0	0	0	0	0	0	0	0	0	0	0
238		0	0	0	0	0	0	0	0	0	0	0	0	0
239		0	0	0	0	0	0	0	0	0	-	0	0	0
240		0	0	0	0	0	0	0	0	0	0	-	0	0

[redacted]

study completed 2013

Product Number	% Copper Gluconate	Product Type	HRIPT Test Yes/No	Occlusivity	Completed Subjects	Did formula induce an allergic response
1	0.2	rinse-off	YES	OCCLUSIVE	217	NO

Product Number 1

Calculation of Amount of Copper Gluconate in mg/cm2	
Concentration of Copper Gluconate in Product in %	0.2
Amount of Product applied to Skin during HRIPT in ml/mg	0.2
Patch Size cm^2	4
Dose density of product applied to patched skin in mg/cm2	50
Dose Density of Copper Gluconate applied to patch skin in mg/cm2	0.10000000

ICDRG Reading scale	
0	No Visible Reaction
±	Faint Minimal Erythema
1	Erythema
2	Intense Erythema, Induration
3	Intense Erythema, Induration, Vesicles
4	Severe reaction with Erythema, Induration, Vesicles (may be weeping)
E	Edema
–	No reading

Details of Test methodolgy and Results	
0	panelist discontinued due to test material reactions
24 hrs	patch duration
9	induction patches
3	weeks induction
2	week rest period
virgin site	challenge
24, 48, 72, 96 hrs post patching	Challenge readings

Grading Scale interpretation	
Low Level Reactions	1
High Level Reaction	2 and above

[redacted]

Baby RO 0.2%
Copper Gluconate

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
1		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
3		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
5		0	0	0	0	0	0	0	0	0	0	0	0	0
6		0	0	0	0	0	0	0	0	0	0	0	0	0
7		0	0	0	0	0	0	0	0	0	0	0	0	0
8		0	0	0	0	0	0	0	0	0	0	0	0	0
9		0	0	0	0	0	0	0	0	0	0	0	0	0
10		0	0	0	0	0	0	0	0	0	0	0	0	0
11		0	0	0	0	0	0	0	0	0	0	0	0	0
12		0	0	0	0	0	0	0	0	0	0	0	0	0
13		0	0	0	0	0	0	0	0	0	0	0	0	0
14		0	0	0	0	0	0	0	0	0	0	0	0	0
15		X	X	X	X	X	X	X	X	X	X	X	X	X
16		0	0	0	0	0	0	0	0	0	0	0	0	0
17		0	0	0	0	0	0	0	0	0	0	0	0	0
18		0	0	0	0	0	0	0	0	0	0	0	0	0
19		0	0	0	0	0	0	0	0	0	0	0	0	0
20		0	0	0	0	0	0	0	0	0	0	0	0	0
21		0	0	0	0	0	0	0	0	0	0	0	0	0
22		0	0	0	0	0	0	0	0	0	0	0	0	0
23		0	0	0	0	0	0	0	0	0	0	0	0	0
24		0	0	0	0	0	0	0	0	0	0	0	0	0
25		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
26		0	0	0	0	0	0	0	0	0	0	0	0	0
27		0	0	0	0	0	0	0	0	0	0	0	0	0
28		0	0	0	0	0	0	0	0	0	0	0	0	0
29		0	0	0	0	0	0	0	0	0	0	0	0	0
30		0	0	0	0	0	0	0	0	0	0	0	0	0
31		0	0	0	0	0	0	0	0	0	0	0	0	0
32		0	0	0	0	0	0	0	0	0	0	0	0	0
33		0	0	0	0	0	0	0	0	0	0	0	0	0
34		0	0	0	0	0	0	0	0	0	0	0	0	0
35		0	0	0	0	0	0	0	0	0	0	0	0	0
36		0	0	0	0	0	0	0	0	0	0	0	0	0
37		0	0	0	0	0	0	0	0	0	0	0	0	0
38		0	0	0	0	0	0	0	0	0	0	0	0	0
39		0	0	0	0	0	0	0	0	0	0	0	0	0
40		0	0	0	0	0	0	0	0	0	0	0	0	0
41		0	0	0	0	0	0	0	0	0	0	0	0	0
42		0	0	0	0	0	0	0	0	0	0	0	0	0
43		0	X	X	X	X	X	X	X	X	X	X	X	X
44		0	X	X	X	X	X	X	X	X	X	X	X	X
45		0	0	0	0	0	0	0	0	0	0	0	0	0
46		0	0	0	0	0	0	0	0	0	0	1	±	±
47		0	0	0	0	0	0	0	0	0	0	0	0	0
48		0	0	0	0	0	0	0	0	0	0	0	0	0
49		0	0	0	0	0	0	0	0	0	0	0	0	0
50		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
51		0	0	0	0	0	0	0	0	0	0	0	0	0
52		0	0	0	0	0	0	0	0	0	0	0	0	0
53		0	0	0	0	0	0	0	0	0	0	0	0	0
54		0	0	0	0	0	0	0	0	0	0	0	0	0
55		0	0	0	0	0	0	0	±	0DR	0	0	0	0
56		X	X	X	X	X	X	X	X	X	X	X	X	X
57		0	0	0	0	0	0	0	0	0	0	0	0	0
58		0	0	0	0	0	0	0	0	0	0	0	0	0
59		0	0	0	0	0	0	0	0	0	0	0	0	0
60		0	0	0	0	0	0	0	0	0	0	0	0	0
61		0	0	0	0	0	0	0	0	0	0	0	0	0
62		0	0	0	0	0	0	0	0	0	0	0	0	0
63		0	0	0	0	0	0	0	0	0	0	0	0	0
64		0	X	X	X	X	X	X	X	X	X	X	X	X
65		0	0	0	0	0	0	0	0	0	0	0	0	0
66		0	0	0	0	0	0	0	0	0	0	0	0	0
67		0	0	0	0	0	0	0	0	0	0	0	0	0
68		0	0	0	0	0	0	0	0	0	0	0	0	0
69		0	0	0	0	0	0	0	0	0	0	0	0	0
70		0	0	0	0	0	0	0	0	0	0	0	0	0
71		0	0	0	0	0	0	0	0	0	0	0	0	0
72		0	0	0	0	0	0	±	±	±	0	0	0	0
73		0	0	0	0	0	0	0	0	0	0	0	0	0
74		0	0	0	0	0	0	0	0	0	0	0	0	0
75		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	Induction Reading									Challenge Reading			
	1	2	3	4	5	6	7	8	9	1	2	3	4
76	0	0	0	0	0	0	0	0	0	0	0	0	0
77	0	0	0	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0	0	0	0
81	0	0	0	0	0	0	0	0	0	0	0	0	0
82	X	X	X	X	X	X	X	X	X	X	X	X	X
83	0	0	0	0	0	0	0	0	0	0	0	0	0
84	0	0	0	0	0	0	0	0	0	0	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	0	0
86	0	0	0	0	0	0	0	0	0	0	0	0	0
87	0	0	0	0	0	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	0	0	0	0	0	0
89	0	0	0	0	0	0	0	0	0	X	X	X	X
90	0	0	0	0	0	0	0	X	X	X	X	X	X
91	0	0	0	0	0	0	0	0	0	0	0	0	0
92	0	0	0	0	0	X	X	X	X	X	X	X	X
93	0	0	0	0	0	X	X	X	X	X	X	X	X
94	0	0	0	0	0	0	0	0	0	0	0	0	0
95	0	0	0	0	0	0	0	0	0	0	0	0	0
96	0	0	0	0	0	0	0	0	0	0	±	0	0
97	0	0	0	0	0	0	0	0	0	0	±	0	0
98	0	0	0	0	0	0	0	0	0	0	0	0	0
99	0	0	0	0	0	0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
101		0	0	0	0	0	0	0	0	0	0	±	1	1
102		0	0	0	0	0	0	0	0	0	0	0	0	0
103		0	0	0	0	0	0	0	0	0	0	0	0	0
104		0	0	0	0	0	0	0	0	0	0	0	0	0
105		0	0	X	X	X	X	X	X	X	X	X	X	X
106		0	0	0	0	0	X	X	X	X	X	X	X	X
107		0	0	0	0	0	0	0	0	0	0	0	0	0
108		0	0	0	0	0	0	0	0	0	0	0	0	0
109		0	0	0	0	0	0	0	0	0	0	0	0	0
110		0	0	0	0	0	0	0	0	0	0	0	0	0
111		0	0	0	0	0	0	0	0	0	0	0	0	0
112		0	0	0	0	0	0	0	0	0	0	0	0	0
113		0	0	0	0	0	0	0	0	0	0	0	0	0
114		0	0	0	0	0	0	0	0	0	0	0	0	0
115		0	0	0	0	0	0	0	0	0	0	0	0	0
116		0	0	0	0	0	0	0	0	0	0	0	0	0
117		0	0	0	0	0	0	0	0	0	0	0	0	0
118		0	0	0	0	0	0	0	0	0	0	0	0	0
119		0	0	0	0	0	0	0	0	0	0	0	0	0
120		0	0	0	0	0	0	0	0	0	0	0	0	0
121		0	0	0	0	0	0	0	0	0	0	0	0	0
122		0	0	0	0	0	0	0	0	0	0	0	0	0
123		0	0	0	0	0	0	0	0	0	0	0	0	0
124		0	0	0	0	0	0	0	0	0	0	0	0	0
125		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
126		0	0	0	0	0	0	0	0	0	X	X	X	X
127		0	0	X	X	X	X	X	X	X	X	X	X	X
128		0	0	0	0	0	0	0	0	0	0	0	0	0
129		0	0	0	0	0	0	0	0	0	0	0	0	0
130		±	±	0	0	0	0	0	0	0	0	0	0	0
131		0	0	0	0	0	0	0	0	0	0	0	X	X
132		0	0	0	0	0	0	0	0	0	0	0	0	0
133		0	0	0	0	0	0	0	0	0	0	0	0	0
134		0	0	0	0	0	0	0	0	0	0	0	0	0
135		0	0	0	0	0	0	0	0	0	0	0	0	0
136		0	0	0	0	0	0	0	0	0	0	1	±	±
137		0	0	0	0	0	0	0	0	0	0	0	0	0
138		0	0	0	0	0	0	0	0	0	0	0	0	0
139		0	0	0	0	0	0	0	0	0	X	X	X	X
140		0	0	0	0	0	0	0	0	0	0	0	0	0
141		0	0	0	0	0	0	0	0	0	0	0	0	0
142		0	0	0	1	±	±	0	0	0	X	X	X	X
143		0	0	0	0	0	0	0	0	0	0	0	0	0
144		0	0	0	0	0	0	0	0	0	0	0	0	0
145		0	0	0	0	0	0	0	0	0	0	0	0	0
146		0	0	0	0	0	0	0	0	0	0	0	0	0
147		0	0	0	0	0	0	0	0	0	0	0	0	0
148		0	0	0	0	0	0	0	0	0	0	0	0	0
149		0	0	0	0	0	0	0	0	0	0	0	1	±
150		0	0	0	X	X	X	X	X	X	X	X	X	X

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
151		0	0	0	0	0	0	0	0	0	0	0	0	0
152		0	0	0	0	0	0	0	0	0	0	0	0	0
153		0	0	0	0	0	0	0	0	0	0	0	0	0
154		0	0	0	0	0	0	0	0	0	0	0	0	0
155		0	0	0	0	0	0	0	0	0	0	0	0	0
156		0	0	0	0	0	0	0	0	0	0	0	0	0
157		0	0	0	0	0	0	0	0	0	0	0	0	0
158		0	0	0	0	0	0	0	0	0	0	0	0	0
159		0	0	0	0	0	0	0	0	0	0	0	0	0
160		0	0	0	0	0	0	0	0	0	0	0	0	0
161		0	0	0	0	0	0	0	0	0	0	0	0	0
162		0	0	0	0	0	0	0	0	0	0	0	0	0
163		0	0	0	0	0	0	0	0	0	0	0	0	0
164		0	0	0	0	0	0	0	0	0	0	0	0	0
165		0	0	0	0	0	0	0	0	0	0	0	0	0
166		0	0	0	0	0	0	0	0	0	0	0	0	0
167		0	0	0	0	0	0	0	0	0	0	0	0	0
168		0	0	0	0	0	0	0	0	0	0	0	0	0
169		0	0	0	0	0	0	0	0	0	0	0	0	0
170		0	0	0	0	0	0	0	0	0	0	0	0	0
171		0	0	0	0	0	0	0	0	0	0	0	0	0
172		0	0	0	0	0	0	0	0	0	0	0	0	0
173		0	0	0	0	0	0	0	0	0	0	0	0	0
174		0	0	0	0	0	0	0	0	0	0	0	0	0
175		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
176		0	0	0	0	0	0	0	0	0	0	0	0	0
177		0	0	0	0	0	0	0	0	0	0	0	0	0
178		0	0	0	0	0	0	0	0	0	0	0	0	0
179		0	0	0	0	0	0	0	0	0	0	0	0	0
180		0	0	0	0	0	0	0	0	0	0	0	0	0
181		0	0	0	0	0	0	0	0	0	0	0	0	0
182		0	0	0	0	0	0	0	0	0	0	0	0	0
183		0	0	0	0	0	0	0	0	0	0	0	0	0
184		0	0	0	0	0	0	0	0	0	0	0	0	0
185		0	0	0	0	0	0	0	0	0	0	0	0	0
186		0	0	0	0	0	0	0	0	0	0	0	0	0
187		0	0	0	0	0	X	X	X	X	X	X	X	X
188		±	±	±	0	0	0	0	0	0	0	0	0	0
189		0	0	0	0	0	0	0	0	0	X	X	X	X
190		0	0	0	0	0	0	0	0	0	0	0	0	0
191		0	1	±	0	0	0	0	0	0	0	0	0	0
192		0	0	0	0	0	0	0	0	0	0	0	0	0
193		0	0	0	0	0	0	0	0	0	0	0	0	0
194		0	0	0	0	0	0	0	0	0	0	0	0	0
195		0	0	0	0	0	0	0	0	0	0	0	0	0
196		0	0	0	0	0	0	0	0	0	0	0	0	0
197		0	0	0	0	0	0	0	0	0	0	0	0	0
198		0	0	0	0	0	0	0	0	0	0	0	0	0
199		0	0	0	0	0	0	0	0	0	0	0	0	0
200		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
201		0	0	0	0	0	0	0	0	0	0	0	0	0
202		0	0	0	0	0	0	0	0	0	0	0	0	0
203		0	0	0	0	0	0	0	0	0	0	0	0	0
204		0	0	X	X	X	X	X	X	X	X	X	X	X
205		0	0	0	0	0	0	0	0	0	X	X	X	X
206		0	0	0	0	0	0	0	0	0	0	0	0	0
207		X	X	X	X	X	X	X	X	X	X	X	X	X
208		0	0	0	0	0	0	0	0	0	0	0	0	0
209		0	0	0	0	0	0	0	0	0	0	0	0	0
210		0	0	0	0	0	0	0	0	0	0	0	0	0
211		0	0	0	0	0	0	0	0	0	0	0	0	0
212		0	0	0	0	0	0	0	0	0	0	0	0	0
213		0	0	0	0	0	0	0	0	0	0	0	0	0
214		0	0	0	0	0	0	0	0	0	0	0	0	0
215		0	0	0	0	0	0	0	0	0	0	0	0	0
216		0	0	0	0	0	0	0	0	0	0	0	0	0
217		0	0	0	0	0	0	0	0	0	0	0	0	0
218		0	0	0	0	0	0	0	0	0	0	0	0	0
219		0	0	0	0	0	0	0	0	0	0	0	0	0
220		0	0	0	0	0	0	0	0	0	0	0	0	0
221		0	0	0	0	0	0	0	0	0	0	0	0	0
222		±	±	0	±	±	0	±	0	0	0	±	±	0
223		0	0	0	0	0	0	0	0	0	0	0	0	0
224		0	0	0	0	0	0	0	0	0	0	0	0	0
225		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
226		0	0	0	0	0	0	0	0	0	0	0	0	0
227		0	0	0	0	0	0	0	0	0	0	0	0	0
228		0	0	0	0	0	0	0	0	0	0	0	0	0
229		±	±	0	0	0	0	0	0	0	0	0	0	0
230		0	0	0	0	0	0	0	0	0	0	0	0	0
231		0	0	0	0	0	0	0	0	0	0	0	0	0
232		0	0	0	0	0	0	0	0	0	0	0	0	0
233		0	0	0	0	0	0	0	0	0	0	0	0	0
234		0	0	0	0	0	0	0	0	0	0	0	0	0
235		0	0	0	0	0	0	0	0	0	0	0	0	0
236		0	0	0	0	0	0	0	0	0	0	0	0	0
237		0	0	0	0	0	0	0	0	0	0	0	0	0
238		0	0	0	0	0	0	0	0	0	0	0	0	0
239		0	0	0	0	0	0	0	0	0	0	0	0	0
240		0	0	0	0	1	±	±	±	±	0	0	0	0

 Study completed 2010

Product Number	% Copper Gluconate	Product Type	HRIPT Test Yes/No	Occlusivity	Completed Subjects	Did formula induce an allergic response
1	0.00008	Rinse Off	YES	OCCLUSIVE	211	NO

Product Number 1

Calculation of Amount of Copper Gluconate mg/cm2	
Concentration of Copper Gluconate in Product in %	0.00008
Amount of Product applied to Skin during HRIPT in ml/mg	0.2
Patch Size cm^2	4
Dose density of product applied to patched skin in mg/cm2	50
Dose Density of Copper Gluconate applied to patch skin in mg/cm2	0.000040

ICDRG Reading scale	
0	No Visible Reaction
±	Faint Minimal Erythema
1	Erythema
2	Intense Erythema, Induration
3	Intense Erythema, Induration, Vesicles
4	Severe reaction with Erythema, Induration, Vesicles (may be weeping)
E	Edema

_	No reading

Details of Test methodolgy and Results	
0	panelist discontinued due to test material reactions
24 hrs	patch duration
9	induction patches
3	weeks induction
2	week rest period
virgin site	challenge
24, 48, 72, 96 hrs post patching	Challenge readings

Grading Scale interpretation	
Low Level Reactions	1
High Level Reaction	2 and above

[redacted]

Adult RO 0.00008%
Copper Gluconate

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

Sub	[redacted]	Induction Reading									Challenge Reading			
		1	2	3	4	5	6	7	8	9	1	2	3	4
001		0	0	0	0	0	0	0	0	0	0	0	0	0
002		0	0	0	0	0	0	0	0	0	0	0	0	0
003		0	0	0	0	0	0	0	0	0	0	0	0	0
004		0	0	0	0	0	0	0	0	0	0	0	0	0
005		0	0	0	0	0	0	0	0	0	0	0	0	0
006		0	0	0	0	0	0	0	0	0	0	0	0	0
007		0	0	0	X	X	X	X	X	X	X	X	X	X
008		0	0	0	0	0	0	0	0	0	X	X	X	X
009		0	0	0	0	0	0	0	0	0	0	0	0	0
010		0	0	0	0	0	0	0	0	0	0	0	0	0
011		0	0	0	0	0	0	0	0	0	0	0	0	0
012		0	0	0	0	0	0	0	0	0	0	0	0	0
013		0	0	0	0	0	0	0	0	0	0	0	0	0
014		0	0	0	0	0	0	0	0	0	0	0	0	0
015		0	0	0	0	0	0	0	0	0	0	0	0	0
016		0	0	0	X	X	X	X	X	X	X	X	X	X
017		0	0	0	0	0	0	0	0	0	0	0	0	0
018		0	0	0	0	0	0	0	0	0	0	0	0	0
019		0	0	0	0	0	0	0	0	0	0	0	0	0
020		0	0	0	0	0	0	0	0	0	0	0	0	0
021		0	0	0	0	0	0	0	0	0	0	0	0	0
022		0	0	0	0	0	0	0	0	0	0	0	0	0
023		0	0	0	0	0	0	0	0	0	0	0	0	0
024		0	0	0	0	0	0	0	0^	0^	0	0	0	-
025		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

		Induction Reading									Challenge Reading			
Sub	[redacted]	1	2	3	4	5	6	7	8	9	1	2	3	4
026		0	0	0	0	0	0	0	0	0	0	0	0	0
027		0	0	0	0	0	0	0	0	0	0	0	0	0
028		0	0	0	0	0	0	0	0	0	0	0	0	0
029		0	0	0	0	0	0	0	0	0	0	0	0	0
030		0	0	0	0	0	0	0	0	0	0	0	0	-
031		0	0	0	0	0	0	0	0	0	0	0	0	0
032		0	0	0	0	0	0	0	0	0	0	0	0	0
033		0	0	0	0	0	0	0	0	0	0	0	0	0
034		0	0	0	0	0	0	X	X	X	X	X	X	X
035		0	0	0	0	0	0	X	X	X	X	X	X	X
036		X	X	X	X	X	X	X	X	X	X	X	X	X
037		0	0	0	0	0	0	0	0	0	0	0	0	0
038		0	0	0	0	0	0	0	0	0	0	0	0	0
039		0	0	0	0	0	0	0	0	0	0	0	0	0
040		0	0	0	0	0	0	0	0	0	0	0	0	0
041		0	0	0	0	0	0	0	0	0	0	0	0	0
042		0	0	0	0	0	0	0	0	±	0	0	0	0
043		0	0	0	0	0	0	0	0	0	0	0	0	0
044		0	0	0	0	0	0	0	0	0	0	0	0	0
045		0	X	X	X	X	X	X	X	X	X	X	X	X
046		0	0	0	0	0	0	0	0	0	0	0	0	0
047		0	0	0	0	0	0	0	0	0	0	0	0	0
048		0	0	0	0	0	0	0	0DR^	0^	0	0	0	0
049		0	0	0	0	X	X	X	X	X	X	X	X	X
050		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

		Induction Reading									Challenge Reading			
Sub	[redacted]	1	2	3	4	5	6	7	8	9	1	2	3	4
051		0	0	0	0	0	0	0	0	0	0	0	0	0
052		0	0	0	0	0	0	0	0	0	0	0	0	0
053		0	0	0	0	0	0	0	0	0	0	0	0	0
054		0	0	0	0	0	0	0	0	0	0	0	0	0
055		0	0	0	0	0	0	0	0	0	0	0	0	0
056		0	0	0	0	0	0	0	0	0	0	0	0	0
057		0	0	0	0	0	0	0	0	0	0	0	0	0
058		0	0	0	0	0	0	0	0	0	0	0	0	0
059		0	0	0	0	±	0	0	0	±	0	0	0	0
060		0	0	0	0	0	0	0	0	0	0	0	0	0
061		0	0	0	0	0	0	0	0	0	0	0	0	0
062		0	0	0	0	0	0	0	0	0	0	0	0	0
063		0	0	0	0	0	0	0	0	0	0	X	X	X
064		0	0	0	0	0	0	0	0	0	0	±	0	0
065		0	0	0	0	0	0	0	0	0	0	0	0	0
066		0	X	X	X	X	X	X	X	X	X	X	X	X
067		0	0	0	0	0	0	0	0	0	0	0	0	0
068		0	0	0	0	0	0	0	0	0	0	0	0	0
069		0	0	0	0	0	0	0	0	0	0	0	0	0
070		0	0	0	0	0	0	0	0	0	0	0	0	0
071		0	0	0	0	0	0	0	0	0	0	0	0	0
072		0	0	0	0	0	0	0	0	0	0	0	0	0
073		0	0	0	0	0	0	0	0	0	0	0	0	0
074		0	0	0	0	0	0	0	0	0	0	0	0	0
075		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

		Induction Reading									Challenge Reading			
Sub	[redacted]	1	2	3	4	5	6	7	8	9	1	2	3	4
076		0	0	0	0	0	0	0	0	0	0	0	0	0
077		0	0	0	0	0	0	0	0	0	0	0	0	0
078		0	0	0	0	0	0	0	0	0	0	0	0	0
079		0	0	0	0	0	0	0	0	±	0	0	0	0
080		0	0	0	0	0	0	0	0	0	0	0	0	0
081		0	0	0	0	0	0	0	0	0	0	0	0	0
082		0	0	0	0	0	0	0	0	0	0	0	0	0
083		0	0	0	0	0	0	0	0	0	0	0	0	0
084		0	0	X	X	X	X	X	X	X	X	X	X	X
085		0	0	0	0	0	0	0	0	±	0	0	0	0
086		0	X	X	X	X	X	X	X	X	X	X	X	X
087		0	0	0	0	0	0	0	0	0	0	0	0	0
088		0	0	0	0	0	0	0	0	0	0	0	0	0
089		0	0	0	0	0	0	0	0	0	0	0	0	0
090		0	0	X	X	X	X	X	X	X	X	X	X	X
091		0	0	0	0	0	0	0	0	0	0	0	0	0
092		0	0	1	1	0	0	0	0	0	0	0	0	0
093		0	0	0	0	0	0	0	0	0	0	0	0	0
094		0	0	0	0	0	0	0	0	0	0	0	0	0
095		0	0	0	0	0	0	0	0	0	0	0	0	0
096		0	0	0	0	0	0	0	0	0	0	0	0	0
097		0	0	0	0	0	0	0	0	0	0	0	0	0
098		0	0	0	0	0	0	0	0	0	0	0	0	0
099		0	0	0	0	0	0	0	0	0	0	0	0	0
100		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

		Induction Reading									Challenge Reading			
Sub	[redacted]	1	2	3	4	5	6	7	8	9	1	2	3	4
101		0	0	0	0	0	0	0	0	0	0	0	0	0
102		0	0	0	0	0	0	0	0	0	0	0	0	0
103		0	0	0	0	0	0	0	0	0	0	0	0	0
104		0	0	0	0	0	0	0	0	0	0	0	0	0
105		0	0	0	0	0	0	0	0^	0^	0	0	0	0
106		X	X	X	X	X	X	X	X	X	X	X	X	X
107		0	0	0	0	0	0	0	0	±	0	0	0	0
108		0	0	0	0	0	0	0	0	0	0	0	0	0
109		0	0	0	0	0	0	0	0	0	0	0	0	0
110		0	0	0	0	0	0	0	0	0	0	0	0	0
111		0	0	0	0	0	0	0	0	0	0	0	0	0
112		0	0	0	0	0	0	0	0	0	0	0	0	0
113		0	0	0	0	0	0	0	0	0	0	0	0	0
114		0	0	0	0	0	0	0	0	0	0	0	0	0
115		0	0	0	0	0	0	0	0	0	0	0	0	0
116		0	0	0	0	0	0	0	0	0	0	0	0	0
117		0	0	0	0	0	0	0	0	0	0	0	0	0
118		0	0	0	0	0	0	0	0	0	0	0	0	0
119		0	0	0	0	0	0	0	0	0	0	0	0	0
120		0	0	0	0	0	0	0	0	0	0	0	0	-
121		0	0	0	0	0	0	0	0	0	0	0	0	0
122		±	0	0	0	0	0	0	±	±	0	0	0	0
123		0	0	0	0	0	0	0	0	0	0	0	0	0
124		0	0	0	0	0	0	0	0	0	0	0	0	0
125		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

		Induction Reading									Challenge Reading			
Sub	[redacted]	1	2	3	4	5	6	7	8	9	1	2	3	4
126		0	0	0	0	0	0	0	0	0	0	0	0	0
127		0	0	0	0	0	0	0	0	0	0	0	0	0
128		X	X	X	X	X	X	X	X	X	X	X	X	X
129		0	0	0	0	0	0	0	0	0	0	0	0	0
130		0	0	0	0	0	0	0	0	0	0	0	0	0
131		0	0	0	0	0	0	0	0	0	0	0	0	0
132		0	0	0	0	0	0	0	0	0	0	0	0	0
133		0	0	0	0	0	0	0	0	0	0	0	0	0
134		0	0	0	0	0	0	0	0	0	0	0	0	0
135		0	0	0	0	0	0	0	0	0	0	0	0	0
136		0	0	0	0	0	0	0	0	0	0	0	0	0
137		0	0	0	0	0	0	0	0	0	0	0	0	0
138		0	0	0	0	0	0	0	0	0	0	0	0	0
139		0	0	0	0	0	0	X	X	X	X	X	X	X
140		0	0	0	0	0	0	0	0	0	0	0	0	0
141		0	0	0	0	0	0	0	0	0	0	0	-	0
142		0	0	0	0	0	0	0	0	0	0	0	0	0
143		0	0	0	0	0	0	0	0	0	0	0	0	0
144		0	0	0	0	0	0	0	0	0	0	0	0	0
145		0	0	0	0	0	0	0	0	0	0	0	0	0
146		0	0	0	0	0	0	0	0	0	0	0	0	0
147		0	0	0	0	0	0	0	X	X	X	X	X	X
148		0	0	0	0	0	0	0	0	1	0	0	0	0
149		0	0	0	0	0	0	0	0	0	0	0	0	0
150		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

		Induction Reading									Challenge Reading			
Sub	[redacted]	1	2	3	4	5	6	7	8	9	1	2	3	4
151		0	0	0	0	0	0	0	0	0	0	0	0	0
152		0	0	0	0	0	0	0	0	0	0	0	0	0
153		0	0	0	1	±	±	±	0	0	0	0	0	0
154		0	0	0	0	0	0	0	0	0	X	X	X	X
155		0	0	0	0	0	0	0	0	0	0	0	0	0
156		0	0	0	0	0	0	0	0	0	0	0	0	0
157		0	0	0	0	0	0	0	0	0	0	0	0	0
158		0	0	0	0	0	0	0	0	0	0	0	0	0
159		0	0	0	0	0	0	0	0	0	0	0	0	0
160		0	0	0	0	0	0	0	0	0	0	0	0	0
161		0	0	0	0	0	0	0	0	0	0	0	0	0
162		0	0	0	0	0	0	0	0	0	0	0	0	0
163		0	0	0	0	0	0	0	0	0	0	0	0	0
164		0	0	0	0	0	0	0	0	0	0	0	0	0
165		0	0	X	X	X	X	X	X	X	X	X	X	X
166		0	0	0	0	0	0	0	0	0	0	0	0	0
167		0	0	0	0	0	0	0	0	0	0	0	0	0
168		0	X	X	X	X	X	X	X	X	X	X	X	X
169		0	0	0	0	0	0	0	0	0	0	0	0	0
170		0	0	0	0	0	0	0	0	0	0	0	0	0
171		0	0	0	0	0	0	0	0	0	0	0	0	0
172		0	0	0	0	0	0	0	0	0	0	0	0	0
173		0	X	X	X	X	X	X	X	X	X	X	X	X
174		0	X	X	X	X	X	X	X	X	X	X	X	X
175		1	0	0	±	±	±	±	0DR	0DR	0	0	0	-

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

		Induction Reading									Challenge Reading			
Sub	[redacted]	1	2	3	4	5	6	7	8	9	1	2	3	4
176		0	0	0	0	0	0	0	0	0	0	0	0	0
177		0	0	0	0	0	0	0	0	0	0	0	0	0
178		0	0	0	0	0	0	0	0	0	0	0	0	0
179		0	0	0	0	0	0	0	0	0	0	0	0	0
180		0	0	0	0	0	0	0	0	0	0	0	0	0
181		X	X	X	X	X	X	X	X	X	X	X	X	X
182		0	0	0	0	0	0	0	0	0	0	0	0	0
183		0	0	0	0	0	0	0	0	0	0	0	0	0
184		0	1	±	0	±	±	±	0	1	0	0	0	0
185		0	0	0	0	0	0	0	0	0	0	±	0	0
186		0	0	0	0	0	0	0	0	0	0	0	0	0
187		0	0	X	X	X	X	X	X	X	X	X	X	X
188		0	0	0	0	0	0	0	0	0	0	±	±	±
189		0	0	0	0	0	0	0	0	0	0	0	0	0
190		0	0	0	0	0	0	0	0	0	X	X	X	X
191		0	0	0	0	0	0	0	0	0	0	0	0	0
192		0	0	0	0	0	0	0	0	0	0	0	0	0
193		2C	0	0	0	0	0	0	0	0	0	±	-	0
194		0	0	0	0	0	0	0	0	0	0	0	0	0
195		0	0	0	0	0	0	0	0	0	0	0	0	0
196		0	0	0	0	0	0	0	0	0	0	0	0	0
197		0	X	X	X	X	X	X	X	X	X	X	X	X
198		0	0	0	0	0	0	0	0	0	0	0	0	0
199		±	0	0	0	0	±	±	0	0	0	0	0	0
200		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

		Induction Reading									Challenge Reading			
Sub	[redacted]	1	2	3	4	5	6	7	8	9	1	2	3	4
201		0	0	0	0	0	0	0	0	0	-	0	0	0
202		0	0	0	0	0	0	0	0	0	0	0	0	0
203		0	0	0	0	0	0	0	0	0	0	0	0	0
204		0	0	0	0	0	0	0	0	0	0	0	0	0
205		0	0	0	0	0	0	0	0	0	0	±	±	0
206		0	0	0	0	0	0	0	0	0	0	0	0	0
207		0	0	0	0	0	0	0	0	0	0	0	0	0
208		0	0	0	0	0	0	0	0	0	0	0	0	0
209		0	0	0	0	0	0	0	0	0	0	0	0	0
210		0	0	0	0	0	0	0	0	0	0	0	0	0
211		0	0	0	0	0	0	0	0	0	0	0	0	0
212		0	X	X	X	X	X	X	X	X	X	X	X	X
213		0	0	0	0	0	0	0	0	0	0	0	0	0
214		0	0	0	0	0	0	0	0	0	0	0	0	0
215		0	0	0	0	0	0	0	0	0	0	0	0	0
216		0	0	0	0	0	0	0	0	0	0	0	0	-
217		0	0	0	0^	0^	0^	0^	0^	0^	0	0	0	0
218		0	0	0	0	0	0	0	0	0	0	±	1	±
219		0	0	0	0	0	0	0	0	0	0	0	0	0
220		0	0	0	0	0	0	0	0	0	0	0	0	0
221		0	0	0	0	0	0	0	0	0	0	0	0	0
222		0	0	0	0	0	0	0	0	0	0	0	0	0
223		0	0	0	0	0	0	0	0	0	0	0	0	0
224		0	0	0	0	0	0	0	0	0	0	0	0	0
225		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

[redacted]

TABLE II: INDIVIDUAL SUBJECT DATA

(see Scoring System, page 16)

		Induction Reading									Challenge Reading			
Sub	[redacted]	1	2	3	4	5	6	7	8	9	1	2	3	4
226		0	0	0	0	0	0	0	0	0	0	0	0	0
227		0	0	0	0	0	0	0	0	0	0	0	0	0
228		0	0	0	0	0	0	0	0	0	0	0	0	0
229		0	0	0	0	0	0	0	0	0	0	±	0	0
230		0	0	0	0	0	0	0	0	0	0	0	0	0
231		0	0	0	0	0	0	0	0	0	0	0	0	0
232		0	0	0	0	0	0	0	X	X	X	X	X	X
233		0	0	0	0	0	0	0	0	0	X	X	X	X
234		0	0	0	0	0	0	0	0	0	0	0	0	0
235		0	0	0	0	0	0	0	0	0	0	0	0	0
236		0	0	0	0	0	±	0	0	±	0	0	0	0
237		0	0	0	0	0	0	0	±	0	0	0	0	0
238		0	0	0	0	0	0	0	0	0	0	0	0	0
239		0	0	0	0	0	0	0	0	0	0	0	0	0
240		0	0	0	0	0	0	0	0	0	0	0	0	0

[redacted]

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: May 20, 2024

SUBJECT: Copper Gluconate

Anonymous. 2022. Topical application ocular irritation screening assay using the Epiocular™ human cell construct (face cream containing 0.0025% Copper Gluconate).

FINAL REPORT

TOPICAL APPLICATION OCULAR IRRITATION SCREENING ASSAY USING THE EPIOCULAR™ HUMAN CELL CONSTRUCT

face cream containing 0.0025% Copper Gluconate

Laboratory Study Number:

[redacted]

Sponsor Study Number:

[redacted]

Study Completion Date:

20 December 2022

Authors:

[redacted]

Sponsor

[redacted]

Performing Laboratory:

[redacted]

Laboratory Project Number:

[redacted]

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SIGNATURE PAGE

Initiation Date:	25 October 2022
Laboratory Start Date:	31 October 2022
Laboratory Completion Date:	3 November 2022
Completion Date:	20 December 2022
Sponsor's Representative:	[redacted]
Testing Facility:	[redacted]
Archive Location:	[redacted]
Director, Laboratory Services:	[redacted]
Study Director:	[redacted] 20 December 2022 Date

TEST ARTICLE RECEIPT

[redacted]	Sponsor's Designation	Lot/Batch #	Physical Description	Receipt Date	Storage Conditions*
[redacted]	[redacted]	[redacted]	off-white cream	14 September 2022	15 to 30 °C (Room Temp)

* - Protected from exposure to light

Sample Information Table

[redacted]

pH @ 25®C	5.70
Product type	Skin care (Face leave-on)
Sample Weight	1 x 2 fl oz bulk in glass jar
Date sample made	08/16/2022
Expiration date	08/16/2023
Storage condition	Room Temperature

INTRODUCTION

The EpiOcular™ Human Cell Construct was used to assess the potential ocular irritation of the test article. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) conversion assay, which measures the NAD(P)H-dependent microsomal enzyme reduction of MTT (and to a lesser extent, the succinate dehydrogenase reduction of MTT) to a blue formazan precipitate, was used to assess cellular

metabolism after exposure to each test article for various exposure times[1]. The duration of exposure resulting in a 50% decrease in MTT conversion in test article-treated EpiOcular™ human cell constructs, relative to control cultures, was determined (ET_{50}).

MATERIALS AND METHODS

The assay procedures were performed as outlined in the study protocol (See Appendix A).

DEVIATIONS

There was one deviation that occurred during the conducting of this study

The times of MTT termination (isopropanol transfer) were inadvertently not recorded on the day of (2 Nov 2022) for the tissues associated with NC 8 hour exposure. This is a deviation from [redacted] which states that [redacted] for data generated to support a non-clinical study. Data generated at [redacted] shall be: (C) Contemporaneous." The correct times were later recorded on 3 Nov 2022 based on the pre-planned schedule and the times recorded on the plates. Since it is a deviation from documentation and no from the procedure which was executed correctly, therefore this deviation is not expected to have any significant impact on the overall outcome of the study.

RESULTS AND DISCUSSION

Test Article Preparation

The test article, [redacted], was administered to the test system without dilution (neat).

Due to its viscosity, dosing devices (i.e., flat-headed cylinders of slightly less diameter than the inner diameter of the tissue insert) were placed over the dose of the test article to ensure a more even spreading over the surface of the tissues.

Direct MTT Reduction Test

The test article, [redacted], was observed to directly reduce MTT in the absence of viable tissue. Therefore, a killed control experiment was performed. The results of the killed control experiment showed that there was little or no direct MTT reduction in the test article-treated killed

[1] Berridge, M.V., Tan, A.S., McCoy, K.D., Wang, R. (1996) The Biochemical and Cellular Basis of Cell Proliferation Assays That Use Tetrazolium Salts. **Biochemica** 4:14-19.

control compared to the negative control-treated killed controls and the MTT reduction in the test article-treated viable tissue was ascribed to the viable cells.

Test Article & Control Exposures

One hundred microliters of the test article, [redacted], was tested in duplicate EpiOcular™ tissues at 4 exposure times of 4, 8, 16, and 24 hours. The positive control, 100 µL of 0.3% Triton™ X-100, was exposed in duplicate tissues for 5, 20, and 60 minutes. The negative control, 100 µL of sterile, deionized water, was treated in duplicate tissues for 1, 8, and 24 hours.

Residual Test Article

The test article, [redacted], could not be completely removed from the exposed killed control tissues following the rinsing and soaking process after the 4 and 24-hour exposure times. The residual test article prolonged the exposure to the tissues, however; given there was little or no direct MTT reduction in the test article-treated killed control (section of Direction MTT reduction Test), and MTT reduction in the test article-treated viable tissues was ascribe to the viable cells, the ET_{50} result was not affected.

Study Notes

During MTT termination, blisters were observed on the killed control tissue treated with negative control at the 1-hour time point.

Evaluation of Test Results

Table 1 summarizes the ET_{50} for the test article and positive control exposures. Generally, the ET_{50} value for test article was calculated by interpolation of two exposure times, one that showed less than 50% relative survival and one that showed greater than 50% survival. If all of the exposure times showed greater than 50% survival, the ET_{50} value was presented as greater than the maximum exposure time.

Criteria for a Valid Test

The assay results were accepted when: 1) The ET_{50} value of the positive control fell within 2.0 standard deviations of the historical mean, that was 10.9-49.5 minutes at the time the assay performed, and 2) The OD_{570} value for the minimum negative control exposure time of 60 minutes was greater than 1.100.

Table 1
Summary Results of the EpiOcular™ Screening Assay
Assay Date: 2 November 2022

[redacted] Test Article Number	Sponsor's Designation	Conc. (w/v)	ET_{50} (hours)	Irritancy Classification*	pH
[redacted]	[redacted]	100%	>24	Non/minimal	DpH
Positive Control	0.3% Triton™ X-100	NA	24.0 minutes	Moderate	NA

NA – Not Applicable

DpH – Discolored pH paper; a pH was not able to be determined since the test article discolored the pH paper.

* - According to Stern, et al., Toxicology In Vitro, 12, 455-461 (1998) Prediction model.

EPIOCULAR [redacted] BIOASSAY

EXPERIMENT DATE: 3-Nov-22 Study No. [redacted]
TEST MATERIAL: [redacted]
TEST ARTICLE:

ET_{50} = >24 Hours

TRIAL 1
CONCENTRATION: 100%

TIME EXPOSURE (Hours)	PERCENT VIABLE
4	111.1
8	107.2
16	92.2
24	63.7

y = Percent Viable
x = Exposure Time
slope=rise/ run=(y1-y2)/ (x1-x2)
y intercept=y-(slope*x)

	X		Y
1	24.0	1	63.7
2	24.0	2	63.7
3	#DIV/0!	3	50
	slope =		#DIV/0!
	y intercept =		#DIV/0!

[redacted]
CONCENTRATION: 100%
TRIAL 1