Safety Assessment of Copper Gluconate as Used in Cosmetics

Status:	Draft Report for Panel Review
Release Date:	March 4, 2024
Panel Meeting Date:	March 28-29, 2024

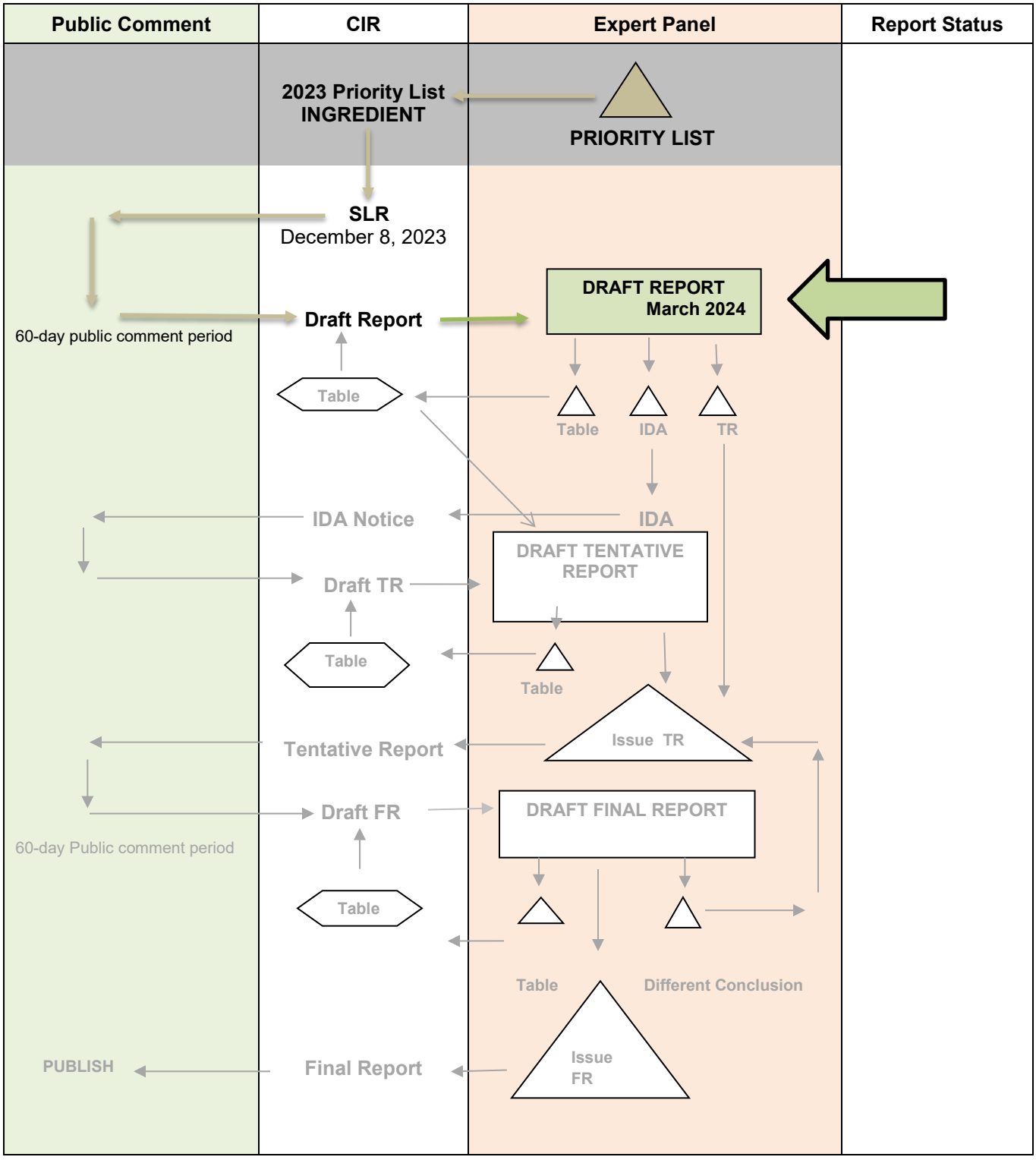
The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D.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

1620 L Street, NW, Suite 1200 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651
cirinfo@cir-safety.org

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Copper Gluconate

MEETING March 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: March 4, 2024
Subject: Safety Assessment of Copper Gluconate as Used in Cosmetics

Enclosed is the Draft Report of the Safety Assessment of Copper Gluconate as Used in Cosmetics (identified as *report_CopperGluconate_032024* in the pdf). This is the first time the Expert Panel for Cosmetic Ingredient Safety (Panel) is seeing a safety assessment of this ingredient. A Scientific Literature Review (SLR) was announced on December 8, 2023.

The Panel has previously reviewed the safety of gluconic acid, potassium gluconate, and sodium gluconate; in 2019, a final report was published with the conclusion that these ingredients are safe in the present practices of use and concentration in cosmetics described in the safety assessment. The full report is available on the CIR website (https://www.cir-safety.org/ingredients).

Mostly data summaries for repeated dose toxicity and developmental and reproductive toxicity were found for this ingredient. Additionally, several quantitative structure-activity relationship (QSAR) models were described in European Chemicals Agency (ECHA) dossiers for these endpoints, and these have been included in this report. CIR staff have also performed an exposure assessment of daily copper exposure resulting from reported use of Copper Gluconate in cosmetics, to be utilized for comparison with the recommended daily allowance and tolerable upper limit values for copper oral intake. ***We welcome the Expert Panel's strategies for utilizing these predictive data in the report.***

Comments on the SLR (*PCPCcomments_CopperGluconate_032024*) that were received from the Council have been addressed and follow this memo. A comments response checklist is also included (*response-PCPCcomments_CopperGluconate_032024*).

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

- 2022 concentration of use data (*data_CopperGluconate_032024*)
- a flow chart (*flow_CopperGluconate_032024*)
- ingredient history (*history_CopperGluconate_032024*)
- search strategy (*search_CopperGluconate_032024*)
- data profile (*dataprofile_CopperGluconate_032024*)-

After reviewing these documents, if the available data are deemed sufficient to make a determination of safety, the Panel should issue a Tentative Report with a safe as used, safe with qualifications, unsafe, or split conclusion, and Discussion items should be identified. If the available data are deemed insufficient, the Panel should issue an Insufficient Data Announcement (IDA), specifying the data needs therein.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: January 11, 2023

SUBJECT: Scientific Literature Review: Safety Assessment of Copper Gluconate as Used in Cosmetics (release date: December 8, 2023)

The Personal Care Products Council respectfully submits the following comments on the Scientific Literature Review Safety Assessment of Copper Gluconate as Used in Cosmetics.

Key Issues

Information about copper being an essential element should be added to this report. A useful resource is the NIH Office of Dietary Supplements factsheet on copper for health professionals at https://ods.od.nih.gov/factsheets/Copper-HealthProfessional/ . The recommended daily allowance (RDA) for copper (900 µg for adults) should be stated in the report. The report should also note the tolerable upper limit (TUL) for copper of 10,000 µg/day which can be found in this 2001 US Institute of Medicine reference https://www.ncbi.nlm.nih.gov/books/NBK222312/ .

Although still in the draft stage, the ATSDR profile (2022) on copper (at https://www.atsdr.cdc.gov/toxprofiles/tp132.pdf) may be helpful to identify additional references on copper. It should be noted that ATSDR did not consider the data sufficient to develop a minimal risk level for copper.

Because copper is an essential element, it does not seem necessary to complete a margin of safety (MoS) calculation for Copper Gluconate. Perhaps a comparison of exposure to copper in cosmetics to the RDA and TUL will help put the exposure to copper from Copper Gluconate in cosmetics in perspective. If the Expert Panel for Cosmetic Ingredient Safety would like MoS calculations included in the report, the products for which the calculations are completed should be those resulting in the highest exposure, rather than the products with the highest concentration. For example, the highest use concentration for moisturizers was 0.0025%. Because moisturizers may be applied over the whole body and left on, they may result in higher exposure than baby shampoo and skin cleansing products, the products for which MoS calculates were completed in the SLR.

Additional Considerations

Abbreviations – CLP should be "Classification, Labelling and Packaging regulations" (as stated in the text in the Short-Term, Subchronic and Chronic section), rather than "guideline" (as stated in Abbreviations)

Impurities – The purity of food grade Copper Gluconate should also be stated (98-102%) as well as the limit for reducing substances (1%).

Short-Term, Subchronic and Chronic – The text should make it clear that in the 2-week study in Fischer 344 rats, only the liver was examined for gross and histopathological changes (as stated in the protocol column of Table 3).

Short-Term, Subchronic and Chronic; Table 3 – Reference 3 indicates that the beagle study was a one-year study in which interim sacrifices were completed at 6 months (24 weeks). The text and the study duration column of Table 3 incorrectly state that this is a 24-week study. The results column of Table 3 correctly states that rats were dosed for 1 year. It is not clear how liver function was determined and why it was considered "minimal".

Developmental and Reproductive Toxicity; Summary; Table 4 – It is not clear what "spermatic parameters" were examined in reference 19. If it was only sperm levels of oxidative stress markers, this should be clearly stated. Rather than in sperm, the Summary and Table 4 states that the markers were measured in testes tissue. If the measurements were completed in the testes, the text needs to be revised to make this clear.

Dermal Irritation and Sensitization – It should be made clear that the hazard classification applies to copper in the occupational setting. 29CFR1910.1200 states: "This section does not require labeling of the following chemicals: (1910.1200(b)(5)(iii)) Any food, food additive, color additive, drug, cosmetic, or medical or veterinary device or product, including materials intended for use as ingredients in such products (e.g., flavors and fragrances), as such terms are defined in the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 301 et seq.) or the Virus-Serum-Toxin Act of 1913 (21 U.S.C. 151 et seq.), and regulations issued under those Acts, when they are subject to the labeling requirements under those Acts by either the Food and Drug Administration or the Department of Agriculture"

Risk Assessment – As stated above, the MoS calculation for Copper Gluconate is not necessary. If the exposure calculation for the baby shampoo is left in the report, the values used to complete the calculation should be explained more clearly. Although the body weight used is for a child (1-3 years old), the surface area is for an adult and represents half the area of the head and the hands.

Summary – Please add the units in the following: "7.5 [?] copper, and in young Capuchin monkeys"

Summary – In the 90-day oral study that looked at kidney effects, please state the media in which urea, creatine, sodium and potassium were measured.

Table 1 – It would be helpful to also provide the formula weight of copper.

Table 3 – In the Results column of the first study, it would be helpful to make it clear that only the liver was examined.

Table 3, Computational – Since it is not a toxicity study, it is not clear if the computational section should be included in this table. It is also misleading to state that the study was done in rats, that the strain and numbers were not specified, and that the dose/concentration was not specified.

Table 4, Computational – Since it is not a toxicity study, it is not clear if the computational section should be included in this table. It is also misleading to state that the studies were done in rats, that the strain and numbers were not specified, and that the dose/concentration was not specified.

Copper Gluconate - March 28-29, 2024 Panel Meeting – Preethi Raj			
Comment Submitter: Personal Care Products Council **Date of Submission: January 11, 2023 (comments received on SLR posted December 8, 2023)**			
#	**Report section/Comment**	**Response/Action**	**Needs Panel Input**
1	Key Issue: Info on copper being an essential element to be added	- Data from the NIH Office of Dietary Supplements factsheet added to Non-Cosmetic Section	
2	Key Issue: -recommended daily allowance (RDA) for copper needs to be stated -tolerable upper limit (TUL) for copper needs to be stated	- RDA and TUL have been added to the Non-Cosmetic section	
3	Key Issue: -ATSDR 2022 copper profile to be checked for references -should be noted that ATSDR did not consider the data sufficient to develop a minimal risk level for copper	- checked references, especially pertaining to Copper Gluconate - statement re: ATSDR not considering data sufficient to develop a minimal risk level for copper added to the Non-Cosmetic section	
4	Key Issue: -suggestion that MoS calculations may not be necessary since copper is an essential element. Alternatively: -provide a comparison of copper exposure to the RDA and TUL, to put copper exposure from Copper Gluconate in cosmetics in perspective - or, calculate MoS for concentration resulting in the highest level of exposure (e.g., 0.0025% Copper Gluconate in a moisturizer), instead of the current MoS for baby shampoo and skin cleansing products	- Risk Assessment section has been revised by staff	♦
5	Abbreviation: CLP should be "Classification, Labelling, and Packaging regulations	- revised	
6	Impurities: the purity of food grade Copper Gluconate should also be stated (98-102%) as well as the limit for reducing substances (1%)	- added	
7	Short-Term, Subchronic, and Chronic - text should make it clear that in the 2-week study in Fischer 344 rats, only the liver was examined for gross and histopathological changes (as stated in the protocol column of Table 3)	- revised	
8	Short-Term, Subchronic, and Chronic; Table 3 – -reference 3: correct beagle study to reflect that it was a 1-yr study in which interim sacrifices were completed at 6 mo (24 wk) - clarify how liver function was determined and why it was considered 'minimal'	- revised - details on how liver function was determined and considered 'minimal' not provided	
9	DART; Summary; Table 4 – revise text to reflect that the measures of oxidative stress were measured in testes tissue	- revised	
10	Dermal Irr and Sens – make it clear that the hazard classification applies to copper in the occupational setting – based on 29CFR1910.1200	- clarified	
11	Risk Assessment – consider whether the MoS calculation for Copper Gluconate is necessary. If the current MoS for baby shampoo is kept, values used for calculation need further explanation.	- noted	♦

12	Summary – Please add the units in the following: "7.5 [?] copper, and in young Capuchin monkeys"	- revised (mg/d)	
13	Summary – In the 90-d oral study (Georgewill 2015) that looked at kidney effects, please state the media in which urea, creatine, sodium, and potassium were measured	- medium in which these values are measured is unclear	
14	Table 3 – Results: make it clear that only the liver was examined.	- clarified	
15	Table 3; Computational - Since it is not a toxicity study, it is not clear if the computational section should be included in this table. It is also misleading to state that the study was done in rats, that the strain and numbers were not specified, and that the dose/concentration was not specified	- revised	
16	Table 4; Computational - Since it is not a toxicity study, it is not clear if the computational section should be included in this table. It is also misleading to state that the studies were done in rats, that the strain and numbers were not specified, and that the dose/concentration was not specified	- revised	

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 is being presented to the Panel for review.

Copper Gluconate Data Profile* – March 28 - 29, 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" 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 2/06/2024

((((((((((((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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1620 L Street, NW, Suite 1200 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651
cirinfo@cir-safety.org

ABBREVIATIONS

APP	amyloid precursor protein
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
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
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
Gadd45α	growth arrest and DNA damage inducible alpha
GHS	Globally Harmonized System
GRAS	generally recognized as safe
GST-P	glutathione S-transferase placental form
HGF	hepatocyte growth factor
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
MoS	margin of safety
MRL	minimal risk level
mRNA	messenger RNA
MT1a	metallothionein 1a
MT2a	metallothionein 2a
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
TG	test guideline
TGF-β	transforming growth factor-β
TNF-α	tumor necrosis factor alpha
TUL	tolerable upper limit
US	United States
VCRP	Voluntary Cosmetic Registration Program

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* (wINCI; *Dictionary*), this ingredient is reported to function in cosmetics as a skin-conditioning agent.[1]

In 2019, the Expert Panel for Cosmetic Ingredient Safety (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://www.cir-safety.org/ingredients).

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 February 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]

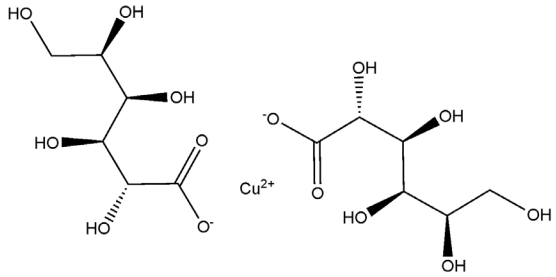

Figure 1. Copper Gluconate

Chemical Properties

Copper Gluconate is a light blue to bluish-green or green solid or crystalline powder that has a formula weight of 453.84 g/mol (compared to 63.55 g/mol atomic weight of copper) and an estimated log K_{ow} of - 2.98.[4-7] 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

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.[8] Additionally, the purity of food-grade Copper Gluconate is 98 - 102%, and the limit for reducing substances is 1%. No further impurities data were found in the published literature, and unpublished data were not submitted.

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).[9] The results of the concentration of use survey conducted by the Council in 2022 indicate that the maximum reported concentration of use for Copper Gluconate in a leave-on formulation is up at 0.006% in eyeliners; overall, the highest maximum reported concentration of use is 0.2% in baby shampoos.[10]

Copper Gluconate is reported to be used in products applied near the eye (at up to 0.006% in eyeliners) and in products that can result in incidental ingestion (e.g., it has 4 reported uses in mouthwashes and breath fresheners and 2 reported uses in lipsticks; concentrations not provided). Copper Gluconate is also reported to be used in other baby products at 0.0005% and at up to 0.2% in baby shampoos. Copper Gluconate is 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).[11]

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.[12] In a 2022 draft toxicological profile, the Agency for Toxic Substances and Disease Registry deemed the oral database was adequate for the derivation of an acute duration oral minimal risk level (MRL; 0.02 mg/kg/d), which was also adopted as the provisional intermediate duration oral MRL. The available data were deemed insufficient for the derivation of an oral chronic MRL and to determine MRLs for all durations of inhalation exposure.[13] The tolerable upper limit (TUL) for copper intake is 10,000 µg/d.[14]

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[15] and as minerals in Annex II of vitamin and mineral substances which may be used in the manufacture of food supplements;[16] 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).[17]

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.[18] The accumulation of copper (dry weight, ng/mg) in the liver, kidney, brain, and heart of the test animals was compared to that of controls (drinking water). There was a statistically significant difference between 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, ng/mg) 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.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

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] The test substance did not classify as toxic in any category. This prediction was based upon Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) Guidance QSAR R6. No further details were provided.

Oral

The acute oral toxicity of Copper Gluconate was tested in Wistar rats, in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 401.[4] Groups of 5 male and 5 female Wistar rats received a single oral dose of 1800, 2400, or 3200 mg/kg bw Copper Gluconate in water (dosage volume of 10 ml/kg bw), via gavage, and were observed for up to 14 d. Most of the animals exhibited apathetic behavior and reduced locomotion 4 h after dosing. These effects were no longer apparent in surviving animals from day 4 of the observation period. All 10 animals in the 3200 mg/kg bw group died within 24 h of dosing; 8 out of 10 of the animals in the 2400 mg/kg group, and 5 out of 10 of the animals in the 2400 mg/kg bw group were found dead within 48 h of dosing. 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. The acute oral LD_{50} was calculated to be 1709 mg/kg bw (males and females combined).

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 3.

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.[19] 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 0.006 or 0.06% (equivalent to mean consumption of 3.46 or 34.9 mg/kg/d, respectively) Copper Gluconate in the diet for 24 wk.[20] 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 0.16% (160 mg/kg/d) Copper Gluconate in the diet for up to 44 wk.[3,21] 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 0.16% 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% (equivalent to 3, 15, or 60 mg/kg/d, respectively) Copper Gluconate in the diet for up to 1 yr.[3,21] 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.[18] The survival curve and lifespan were significantly reduced by 14.7 and 14.4% in the 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.[22] 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, levels of the antibodies Ki67 and MT1 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 summarized below can be found in Table 4.

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.[23] 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.[3,21] 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.[3,21] 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 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.[3,21] 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.

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.[19] 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.[24] 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.[25] 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 the test article was considered nephrotoxic.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Dermal irritation and sensitization studies were not found in the published literature and unpublished data were not submitted. However, QSAR predictions of the primary dermal irritation index (PDII) of Copper Gluconate in rabbit skin and the sensitizing potential of Copper Gluconate in mouse skin, found in an ECHA dossier, are described.[4] Based on REACH guidance, the QSAR model predicted that Copper Gluconate would produce a PDII of 2.26 in rabbit skin. In another QSAR-based prediction described in an ECHA dossier, it was predicted that the effective concentration to induce a 3-fold increase in local lymph node proliferative activity (EC3), in a mouse model was 5.08% Copper Gluconate. 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.[4,26] Furthermore, this GHS hazard classification applies to copper use in the occupational setting, and does not require labeling as a chemical used in a food, food additive, color additive, drug, cosmetic, or medical or veterinary device or product (29CFR1910.121).

OCULAR IRRITATION STUDIES

Ocular irritation studies were not found in the published literature and unpublished data were not submitted. However a QSAR prediction for the modified maximum average score (MMAS) for ocular irritation in rabbit eyes, found in an ECHA dossier, is described.[4] Using QSAR prediction software (QSAR Toolbox 3.4.0.17) and the REACH guideline on QSAR, the MMAS for Copper Gluconate was predicted to be 49.5 in rabbit eyes. The test article 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, the test article 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.[27] 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.[12] 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 employed the in silico tool VERMEER Cosmolife (previously named SpheraCosmolife)[28] to estimate the daily exposure to copper that results from the highest use concentration of Copper Gluconate (e.g., 0.2% in baby shampoos) or for the product category with a higher level of exposure (e.g., 0.0025% in body lotion and 0.1% in make-up remover).

i) Copper Gluconate at 0.2% in baby shampoos

The following parameters, retrieved from the SCCS Notes of Guidance (NoG) for the Testing of Cosmetic Ingredients and Their Safety Evaluation (12th version),[29] have been used in the estimation of daily exposure by VERMEER Cosmolife:

Estimated daily amount applied in baby shampoos: 9.06 g/d = 9060 mg/d
Retention factor: 0.01
Type of exposure: rinse-off
Time of exposure: 0.5 h
Surface area involved: 1440 cm^2 (conservative, utilized an adult's surface area for application)
Body weight considered for the product exposure: baby (1-3 yr, 12 kg)
Relative daily exposure of baby shampoos: 9060 mg/d × 0.01 (retention factor) = 90.6 mg/d
Exposure to Copper Gluconate as used in baby shampoos: 90.6 mg/d × 0.2% (use concentration) = 0.1812 mg/d

It should be noted that VERMEER Cosmolife calculates the daily exposure to baby shampoo using the surface area of an adult (1440 cm^2). According to dermal exposure factors from the U.S. EPA for a baby (3 yr), the total body surface area is 6100 cm^2, with the head's surface area making up 8.4% of the total.[30] It is assumed that half of the baby's head is exposed, leading to a calculated exposure surface area of 256 cm^2. Additionally, according to the SCCS NoG, the average daily shampoo exposure for an adult is 110 mg/d.[29] Based on these parameters, the relative daily exposure of baby shampoos is determined to be 19.6 mg/d. Consequently, the exposure to Copper Gluconate as used in baby shampoos is: 19.6 mg/d × 0.2% (use concentration) = 0.0392 mg/d.

The proportion of copper in Copper Gluconate is approximately 14%; therefore, daily exposure to copper from Copper Gluconate in baby shampoos is 0.0392 mg/d × 14% = 000549 mg/d = 5.49 µg/d
Systemic exposure dose (SED) with 100% absorption (oral absorption): 5.49 µg/d

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

Likewise, the following parameters are retrieved from the SCCS NoG.[29]

Estimated daily amount applied in a make-up remover: 5 g/d = 5000 mg/d
Retention factor: 0.1
Body weight considered for the product exposure: adult (60 kg)
Type of exposure: rinse-off
Time of exposure: 0.5 h
Surface area involved: 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

iii) Copper Gluconate at 0.0025% in moisturizers (e.g., body lotion, leave-on)

The following parameters are retrieved from the SCCS NoG.[29]
Estimated daily amount applied body lotion: 7.82 g/d = 7820 mg/d
Retention factor: 1.0
Body weight considered for the product exposure: adult (60 kg)
Type of exposure: leave-on
Time of exposure: 24 h
Surface area involved: 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 × 0.0025% (use concentration) = 0.1955 mg/d
Daily exposure to copper from Copper Gluconate in body lotion: 0.1955 mg/d × 14% = 0.02737 mg/d = 27.37 µg/d
SED with 100% absorption (oral absorption): 27.37 µg/d

The exposure assessment indicates that the daily exposure to copper from Copper Gluconate in baby shampoos, make-up removers, and body lotions does not exceed 5.49 µg/d, 70 µg/d, and 27.37 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 2022 Council survey data, Copper Gluconate is reported to be used in 170 formulations, 140 of which are leave-ons, and the highest reported concentration of use in a leave-on formulation is at up to 0.006% in eyeliners. Copper Gluconate is also reported to be used in other baby products at 0.0005% and at up to 0.2% in baby shampoos, which is the maximum reported concentration of use. 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. 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.

An acute dermal LD_{50} of 2130 mg/kg Copper Gluconate was predicted for rats, based on a QSAR model. Male and female Wistar rats received a single dose of 1800, 2400, or 3200 mg/kg bw Copper Gluconate, in water, via gavage, in an acute oral toxicity study. All 10 animals in the 3200 mg/kg bw group died within 24 h of dosing; 8 out of 10 of the animals in the 2400 mg/kg group, and 5 out of 10 of the animals in the 2400 mg/kg bw group were found dead within 48 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 0.16% 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 0.16% 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 14.7 and 14.4% in the mid- and high-dose groups, 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.

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.

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. 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.

The in vitro dermal absorption of Copper Gluconate in surgically removed abdominal human skin was predicted using a QSAR model. The calculated dermal absorption value was 1.42% based upon the assumed human weight of 80 kg, with an approximate skin extension of 2 m^2 and an exposure level of 5 mg/cm^2 Copper Gluconate. The deduced absorption level of 17.75 mg/kg Copper Gluconate (based on a worst-case scenario at 5 mg/cm^2) was not considered representative of risk, in comparison to the lethal dermal dose of 2130 mg/kg.

Using the in silico tool, VEERMEER Cosmolife, daily exposures to copper from Copper Gluconate were estimated to not exceed 5.49 µg/d in baby shampoos, 70 µg/d in make-up removers, and 27.37 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).

DISCUSSION

To be developed.

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)	453.84 (compared to 63.55 g/mol atomic weight of copper)	5,7
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 *Soluble* *Insoluble*	 water, alcohol (slightly) acetone, ether, organic solvents	4,5
log K_{ow}	-2.98 (estimated)	4

Table 2. Frequency (2023)[9] and concentration (2022)[10] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)
Totals*	**170**	**0.000025 - 0.2**
summarized by likely duration and exposure**		
Duration of Use		
Leave-On	*140*	*0.0005 - 0.006*
Rinse-Off	*30*	*0.000025 - 0.2*
Diluted for (Bath) Use	*NR*	*NR*
Exposure Type*		
Eye Area	13	0.0005 - 0.006
Incidental Ingestion	6	NR
Incidental Inhalation-Spray	53[a]; 46[b]	0.0005[a]
Incidental Inhalation-Powder	5; 46[b]	0.0005 - 0.003[c]
Dermal Contact	156	0.0001 - 0.1
Deodorant (underarm)	NR	NR
Hair - Non-Coloring	8	0.000025 - 0.2
Hair-Coloring	NR	NR
Nail	NR	NR
Mucous Membrane	8	NR
Baby Products	2	0.0005 - 0.2
as reported by product category		
Baby Products		
Baby Shampoos	2	0.2
Other Baby Products	NR	0.0005
Eye Makeup Preparations		
Eyeliner	NR	0.006
Eye Lotion	7	0.0005
Eye Makeup Remover	1	NR
Other Eye Makeup Preparations	5	NR
Hair Preparations (non-coloring)		
Hair Conditioner	NR	0.000025
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
Personal Cleanliness Products		
Bath Soaps and Detergents	1	NR
Other Personal Cleanliness Products	1	NR
Skin Care Preparations		
Cleansing	17	0.0023 - 0.1
Face and Neck (exc shave)	39	not spray: 0.0005 - 0.003
Body and Hand (exc shave)	7	NR
Moisturizing	35	not spray: 0.0005 - 0.0025
Night	5	not spray: 0.005
Paste Masks (mud packs)	NR	0.0001 - 0.005
Skin Fresheners	7	NR
Other Skin Care Preparations	10	0.0005
Suntan Preparations		
Other Suntan Preparations	1	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. 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.	[19]
Copper Gluconate	feed	Male and female rats (number not specified)	6 mo (24 wk)	0.006 or 0.06% (equivalent to 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.	[20]
Copper Gluconate	feed	Rats (25/sex/group)	Up to 44 wk	0.16% (160 mg/kg/d)	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 0.16% Copper Gluconate in the diet was considered toxic.	[3,21]
Copper Gluconate	feed	Male and female Beagle dogs (6/group/sex)	Up to 1 yr (52 wk)	0.012, 0.06, or 0.24% (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.	[3,21]
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) 2nd experiment: 0.0005 or 0.001 M Copper Gluconate (12.7 or 63.5 ppm copper) amount of water not specified	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. 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. Control groups consumed the same amount of distilled water.	The survival curve, and lifespan, was significantly reduced by 14.4% (0.005 M; $p < 0.01$) for treated mice in the 1st experiment. An 11.8% (0.0005 M; $p > 0.05$) and 14.7% (0.001 M; $p < 0.01$) reduction in lifespan for mice in the 2nd experiment indicated that a dose-response relationship did not exist for survival. Animals that consumed Copper Gluconate weighed slightly less than controls throughout the experiment and died earlier.	[18]

Table 3. 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) 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 the antibodies Ki67 and MT1 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.	[22]
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).	[22]
COMPUTATIONAL							
Copper Gluconate	NA	NA	NA	NA	Results from a QSAR model (described in an ECHA dossier) are 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; LOAEL – lowest-observed-adverse-effect-level; *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 4. 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.	[23]
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.	[3,21]
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.	[3,21]
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.	[3,21]
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

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Concentration of Use by FDA Product Category – Copper Gluconate

Product Category	Maximum Concentration of Use
Baby shampoos	0.2%
Other baby products	0.0005%
Eyeliners	0.006%
Eye lotions	0.0005%
Hair conditioners	0.000025%
Shampoos (noncoloring)	0.000025%
Other makeup preparations	0.0025%
Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	0.0023-0.1%
Face and neck products Not spray	 0.0005-0.003%
Moisturizing products Not spray	 0.0005-0.0025%
Night products Not spray	 0.005%
Paste masks and mud packs	0.0001-0.005%
Other skin care preparations	0.0005%
Other suntan preparations	0.0005%

Information collected in 2022

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