Safety Assessment of Copper Gluconate as Used in Cosmetics

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All interested persons are provided 60 days from the above release date (i.e., February 6, 2024) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party, and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.

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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 guideline
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
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
HGF	hepatocyte growth factor
IL-1α	interleukin 1-alpha
INCHEM	International Programme on Chemical Safety
GST-P	glutathione S-transferase placental form
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
mRNA	messenger RNA
MT1a	metallothionein 1a
MT2a	metallothionein 2a
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
Panel	Expert Panel for Cosmetic Ingredient Safety
PDII	primary dermal irritation index
p21	tumor protein p21
p53	tumor protein p53
QSAR	quantitative-structure activity relationship
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
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 on the safety of monosaccharides, disaccharides, and related ingredients, including 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 October 2023. 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.

Most 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]

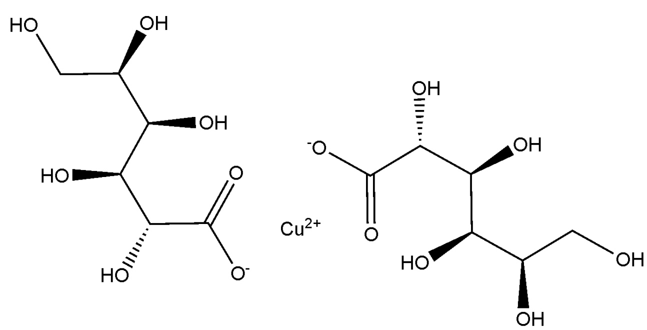

Figure 1. Copper Gluconate

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 and an estimated log K_{ow} of - 2.98.[4-6] 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 that is GRAS 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.[7] No further impurities data were found in the published literature, and unpublished data were not submitted.

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 the 2023 VCRP survey data, Copper Gluconate has 170 reported uses, 140 of which are in leave-on formulations (Table 2).[8] 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.[9]

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).[10]

Non-Cosmetic

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 Gluconate is categorized as a mineral substance in Annex II of vitamin formulations and mineral substances which may be added to foods[11] and as a mineral in Annex II of vitamin and mineral substances which may be used in the manufacture of food supplements;[12] 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).[13]

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.[14] 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 results 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 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, Subchronic, and Chronic Toxicity Studies

Details on the oral and computational short-term, subchronic, and chronic oral toxicity studies 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.[15] No differences in final body weight, liver weight, food consumption, or gross or histological changes were observed in the treated animals, compared to controls. Upon performing gene expression analysis in the liver, hepatic mRNA expression of metallothionein 1a (*Mt1a*; a metal metabolism-related gene) and growth arrest and DNA damage inducible alpha (*Gadd45*α; 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; other gene expression levels were not affected. In another study, 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.[16] Copper content was elevated in the kidneys of animals fed the diet containing 0.06% Copper Gluconate. 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 24 wk.[3,17] Accumulation of copper was seen in the liver, kidneys, and spleen of animals in the 0.24% group; minimal liver function observed in 1 out of 12 dogs in the 0.24% group was reversible within a 12-wk withdrawal period. No other test-article related effects were observed.

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.[3,17] 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. 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.[14] 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.[18] 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 (*NF*κ*B*), hepatocyte growth factor (*HGF)*, and transforming growth factor-β (*TGF*β*))*) 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 and computational developmental and reproductive toxicity studies 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 spermatic parameters in a 90-d study.[19] 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. 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.[3,17] 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.[3,17] 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 reproductive and developmental 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* TA97 and TA102 strains 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.[3,17] 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.[15] 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*, apoptosis-related genes *Gadd45α* and *p21*, inflammation-related genes *TNF*-α (tumor necrosis factor alpha), *IL-1*α (interleukin 1-alpha), *Nos2* (nitric oxide synthase 2), and *c-fos* (protein c-Fos; a proto-oncogene) were significantly increased in the 0.6% group, irrespective of nitrosamine treatment, while *p53* (tumor protein p53; apoptosis-related) 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 a separate short-term study, the male rats (5/group) were fed diets containing 0, 0.001, 0.03, or 0.6% Copper Gluconate for 2 wk. Copper Gluconate treatment did not exert any adverse effects, i.e., no impact was observed on final body weight, liver weight, or food intake; no Copper Gluconate-related alterations, either at a macroscopic or histological level, were observed in the livers of any of the animals. In addition, in the short-term experiment, there was no impact on the expression levels of inflammation-related genes such as *TNF*-α, *IL-1*α, and *Nos2*, nor on the proto-oncogene, *c-fos*. 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.[20] 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.[21] 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 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 Globally Harmonized System (GHS) criteria, the substance was considered as having 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 (29CFR1910.1200)).[22]

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.[23] 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.

RISK ASSESSMENT

A QSAR prediction for the in vitro dermal absorption of Copper Gluconate in surgically removed human abdominal skin, performed in accordance with OECD TG 417 and OECD TG 428, is described in an ECHA dossier.[4] The calculated dermal absorption value was 1.42% based upon an 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. Based on these parameters, it was deduced that the study subject would have an absorption level of 17.75 mg/kg Copper Gluconate (based on a worst-case exposure scenario at 5 mg/cm^2), which, in comparison to the lethal dermal dose of 2130 mg/kg Copper Gluconate, was not considered representative of risk.

As stated earlier in this report, two NOAEL values for Copper Gluconate were calculated using QSAR models, as described in an ECHA dossier: 318 mg/kg bw/d for oral reproductive toxicity in rats and 793 mg/kg bw/d for oral developmental toxicity in rats.[4] The highest maximum reported use concentration of Copper Gluconate is 0.2% in baby shampoos, as indicated in Table 2. For assessing risk, CIR staff employed the in silico tool VERMEER Cosmolife (previously named SpheraCosmolife)[24] to estimate the corresponding systemic toxicity, and subsequently determine the margin of safety (MoS).

i) <u>Copper Gluconate at 0.2% in baby shampoos</u>

VERMEER Cosmolife provides the following parameters, using values retrieved from the SCCS Notes of Guidance (NoG) for the Testing of Cosmetic Ingredients and Their Safety Evaluation (11th version):[25]

Body weight used for the product exposure: baby (1-3 years, 12 kg)
Surface area involved: 1440 cm^2
Type of exposure: rinse-off
Time of exposure: 0.5 h
Relative daily exposure for the selected body weight: 7.55 mg/kg bw/d
Systemic Exposure Dose (SED) with 100% absorption (oral absorption): 0.02 mg/kg bw/d
SED with 50% absorption (dermal absorption): 0.0076 mg/kg bw/d

Using the IRFMN/CORAL model,[24,26] VERMEER Cosmolife predicts an NOAEL of 1687.45 mg/kg bw/d; however, this prediction is considered to have a moderate reliability level. Consequently, the MoS is calculated to be 223,503 at 50% absorption, and 111,752 at 100% absorption. When using the NOAEL value of 318 mg/kg bw/d as described in an ECHA dossier, and combining it with the SED estimated by VERMEER Cosmolife, the resulting MoS is 41,842 for 50% absorption and 15,900 for 100% absorption.

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

Likewise, VERMEER Cosmolife provides the following parameters for makeup remover in the skin cleansing product category (use category for which Copper Gluconate has the highest reported dermal contact concentration) that aligns with the values highlighted in the SCCS NoG:[25]

Body weight used for the product exposure: adult (60 kg)
Surface area involved: 565 cm^2
Type of exposure: rinse-off
Time of exposure: 0.5 h
Relative daily exposure for the selected body weight: 8.33 mg/kg bw/d
SED with 100% absorption (oral absorption): 0.0083 mg/kg bw/d
SED with 50% absorption (dermal absorption): 0.0042 mg/kg bw/d

Based on IRFMN/Coral model,[24,26] VERMEER Cosmolife estimates the NOAEL to be 1687.45 mg/kg bw/d, though it is noted that this prediction has a moderate confidence level. As a result, the derived MOS stands at 405,150 with 50% absorption and 202,575 with 100% absorption. Utilizing the NOAEL value of 318 mg/kg bw/d as predicted by ECHA, in conjunction with the SED estimated by VERMEER Cosmolife, the resulting MOS is 75,714 for 50% absorption and 38,313 for 100% absorption.

The MoS values were estimated for 2 additional uses of Copper Gluconate in skin-cleansing preparations, assuming 50% absorption. The MoS of Copper Gluconate at 0.1% in a shower gel, with a relative daily exposure of 2.79 mg/kg bw/d, is

1,209,642. The MoS of Copper Gluconate at 0.1% in a hand wash soap, with a relative daily exposure of 3.33 mg/kg bw/d, is 1,013,484. All of the MoS values described herein are considered protective.

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. 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. Male and female Beagle dogs (6/sex/group) were administered 0.012, 0.06, or 0.24% Copper Gluconate, in the diet, for 24 wk; 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.

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. 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 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 study, examining effects upon spermatic parameters. 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 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 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 IRFMN/CORAL model, VERMEER Cosmolife predicts an NOAEL of 1687.45 mg/kg bw/d and a computed MoS of 223,503 (assuming 50% absorption) and 111,752 (assuming 100% absorption) for the reported use of 0.2% Copper Gluconate in baby shampoos. When using the NOAEL value of 318 mg/kg bw/d as described in an ECHA dossier, in conjunction with the SED estimated by VERMEER Cosmolife, the resulting MoS is 41,842 for 50% absorption and 15,900 for 100% absorption. Additionally, the computed MoS values for the reported use of Copper Gluconate in skin cleansing preparations (all at a 0.1% concentration and assuming 50% absorption) are: 75,714 for a makeup remover; 1,209, 642 for a shower gel; and 1,013,84 for a hand wash soap.

INFORMATION SOUGHT

The CIR is seeking the following information on the ingredient in this report, as used in cosmetics, for use in the resulting safety assessment:

- Method of manufacture and impurities of this ingredient, as used in cosmetics
- Dermal absorption data
- Dermal irritation and sensitization data, at or above the reported maximum concentration of use
- Toxicological, or other, data that may inform on the cosmetic safety of this ingredient

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	5
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)[8] and concentration (2022)[9] 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 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.	15
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.	16
Copper Gluconate	feed	Male and female Beagle dogs (6/group/sex)	6 mo (24 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. At the end of the experimental period, 2 animals of each sex were killed and necropsied. No further details were provided	No differences were noted in general appearance, behavior, food consumption, or body weight gain, between test animals and controls. After a year 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.	3,17
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.	3,17

Table 3. Repeated dose toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
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.	14
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.	18
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 was 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).	18
				COMPUTATIONAL			
Copper Gluconate	Not specified	Rats (strain and number not specified)	Short-term repeated dose oral toxicity (no further details provided)	Not specified	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-expected-adverse-effect-level; *MT1a* –

metallothionein 1a; *MT2a* – metallothionein 2a; mRNA – messenger ribonucleic acid; *NFκβ* - nuclear factor kappa-light-chain-enhancer of activated B cells; *Nos2* – nitric oxide synthase 2; p53 – tumor protein p53; 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; QSAR – quantitative-structure activity relationship

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.	19
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.	3,17
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.	3,17
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 second 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.	3,17

Table 4. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
COMPUTATIONAL						
Copper Gluconate	Not specified	Rats (strain and number not specified)	Not specified	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	Not specified	Rats (strain and number not specified)	Not specified	as above, but for developmental toxicity in rats	NOAEL = 793 mg/kg bw/d	4

DMSO – dimethyl sulfoxide; NOAEL – no-observed-adverse-effect-level; QSAR - quantitative-structure activity relationship; REACH - Registration, Evaluation, Authorisation, and Restriction of Chemicals

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