Safety Assessment of Hydroxy Tetramethylpiperidine Oxide and Tris(Tetramethylhydroxypiperidinol) Citrate as Used in Cosmetics

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All interested persons are provided 60 days from the above release date (i.e., February 14, 2021) to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to the Cosmetic Ingredient Review (CIR) will be discussed in open meetings, will be available at the CIR office for review by any interested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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ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Hydroxy Tetramethylpiperidine Oxide and Tris(Tetramethylhydroxypiperidinol) Citrate as used in cosmetic formulations. These ingredients are reported to function as an antioxidant and a light stabilizer, respectively. The Panel considered the available data and concluded that Hydroxy Tetramethylpiperidine Oxide and Tris(Tetramethylhydroxypiperidinol) Citrate are safe as used in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This is a safety assessment of Hydroxy Tetramethylpiperidine Oxide and Tris(Tetramethylhydroxypiperidinol) Citrate as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), Hydroxy Tetramethylpiperidine Oxide is reported to function as an antioxidant, and Tris(Tetramethylhydroxypiperidinol) Citrate is reported to function as a light stabilizer, in cosmetics.[1] In 2014, the Expert Panel for Cosmetic Ingredient Safety (Panel) published a safety assessment of a related ingredient, citric acid, and 32 inorganic citric acid salts and alkyl citrate esters, concluding that these ingredients are safe in the present practices of use and concentration in cosmetics.[2]

Hydroxy Tetramethylpiperidine Oxide and Tris(Tetramethylhydroxypiperidinol) Citrate are structurally related as piperidine nitroxides. Therefore, these cosmetic ingredients have been reviewed together in this assessment.

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 exhaustive search of the world's literature. 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 Cosmetic Ingredient Review (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.

Much of the data included in this safety assessment was found on the European Chemicals Agency (ECHA) website.[3,4] Please note that the ECHA website provides summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when ECHA is cited.

CHEMISTRY

Definition and Structure

Hydroxy Tetramethylpiperidine Oxide (CAS No. 2226-96-2) is an organic compound and Tris(Tetramethylhydroxy-piperidinol) Citrate (CAS No. 220410-74-2) is a salt. These piperidine nitroxides conform to the structures shown in Figures 1 and 2, respectively.[1]

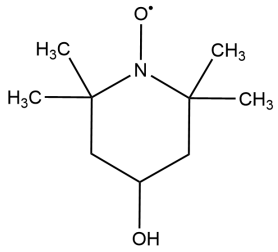

Figure 1. Hydroxy Tetramethylpiperidine Oxide

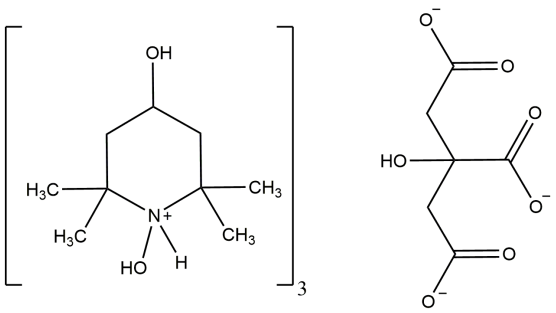

Figure 2. Tris(Tetramethylhydroxypiperidinol) Citrate

Chemical Properties

Hydroxy Tetramethylpiperidine Oxide has a formula weight of 172.24 g/mol and a calculated log P_{ow} of 0.56,[3] while Tris(Tetramethylhydroxypiperidinol) Citrate has a formula weight of 711.9 g/mol and a log P_{ow} of -0.29.[4] Both are soluble in water. The chemical properties of these cosmetic ingredients are further outlined in Table 1.

Method of Manufacture

A general synthesis mechanism for Hydroxy Tetramethylpiperidine Oxide involves derivation from triacetoneamine.[5] Method of manufacture data were not found, or received, for Tris(Tetramethylhydroxypiperidinol) Citrate.

Impurities

Impurities data were not found in the published literature, and unpublished data were not submitted.

USE

Cosmetic

The safety of the cosmetic ingredients 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 these ingredients in cosmetics. Use frequencies of individual ingredients in cosmetics is collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Council, of maximum reported use concentrations by product category.

Frequency of use data were not available for Hydroxy Tetramethylpiperidine Oxide in the VCRP;[6] however, according to a concentration of use survey conducted by the Council in 2020, this ingredient is reported to be used in nail formulations, at a maximum concentration of 12.5% in basecoats and undercoats.[7] According to 2020 VCRP data, Tris(Tetramethylhydroxy-piperidinol) Citrate is reported to be used in 388 cosmetic formulations, most of which are leave-on formulations (335 uses; Table 2).[6] The results of the concentration of use survey conducted by the Council in 2018 indicate that the maximum use concentration of this ingredient in leave-on dermal products is 0.05% in cologne and toilet waters.[8]

Tris(Tetramethylhydroxypiperidinol) Citrate is used in formulations applied to the eye area, at up to 0.005% in eye lotions. It is also used in products which allow for mucous membrane exposure, such as in bath soaps and detergents, at reported maximum concentrations of 0.05%. According to VCRP data, Tris(Tetramethylhydroxypiperidinol) Citrate is used in a baby product formulation; however, concentration of use data were not reported for any baby products.

Additionally, Tris(Tetramethylhydroxypiperidinol) Citrate is used in cosmetic sprays and could possibly be inhaled; for example, Tris(Tetramethylhydroxypiperidinol) Citrate is reported to be used up to 0.05% in cologne and toilet waters. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters > 10 μm, with propellant sprays yielding a greater fraction of droplets/particles < 10 μm compared with pump sprays.[9, 10] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[11, 12]

Hydroxy Tetramethylpiperidine Oxide and Tris(Tetramethylhydroxypiperidinol) Citrate are not restricted from use in any way under the rules governing cosmetic products in the European Union.[13]

Non-Cosmetic

Data for the non-cosmetic use of Tris(Tetramethylhydroxypiperidinol) Citrate were not found. Clinically, Hydroxy Tetramethylpiperidine Oxide has been noted for its potential to function as a nitroxide, to provide protection against radiation and oxidative stresses, both in vitro and in vivo.[14]

TOXICOKINETIC STUDIES

Toxicokinetic studies were not found in the published literature, and unpublished data were not submitted.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The acute dermal, oral, and inhalation toxicity studies summarized below are described in Table 3.

The dermal LD_{50} of Hydroxy Tetramethylpiperidine Oxide was determined to be > 2000 mg/kg bw in male and female Sprague-Dawley rats.[3] The dermal LD_{50} of Tris(Tetramethylhydroxypiperidinol) Citrate was determined to be > 2136 mg/kg bw in male and female New Zealand white rabbits.[4]

The oral LD_{50} of Hydroxy Tetramethylpiperidine Oxide was determined to be 953 mg/kg bw in males, 1115 mg/kg bw in females, and 1053 mg/kg bw in both sexes (combined) in Tif/RAIf rats.[3] In an acute oral toxicity study of Tris(Tetramethyl-

hydroxypiperidinol) Citrate, the LD_{50} was determined to be 2495 mg/kg bw in males, between 1068 and 1602 mg/kg bw in females, and 1758 mg/kg bw in both sexes (combined) in Sprague-Dawley rats.[4]

In an acute inhalation study, performed in accordance with Organisation for Economic Co-operation and Development test guideline (OECD TG) 403, no mortality or gross abnormalities occurred when male and female Sprague-Dawley rats were exposed (nose-only) to aerosolized Tris(Tetramethylhydroxypiperidinol) Citrate, at a concentration of 5.08 mg/l, with a mass median aerodynamic diameter (MMAD) of 3.8 µm, for 4 h.[4] The LC_{50} was determined to be > 5.08 mg/l.

Short-Term Toxicity Studies

Oral

Hydroxy Tetramethylpiperidine Oxide

In accordance with OECD TG 407, groups of 6 male and 6 female Sprague-Dawley rats were administered 0 (vehicle; water), 8, 40, 200, or 1000 mg/kg bw/d Hydroxy Tetramethylpiperidine Oxide, via gavage for 28 d, and then killed.[3] Two additional recovery groups, consisting of 6 males and 6 females that were administered either the vehicle or the highest dose, were kept alive and observed for 14 d after treatment. No mortality occurred and no abnormalities were reported during the recovery period. In the normal test groups, salivation was observed in all animals in the 1000 mg/kg group at varied timepoints of dosing, and in 1 male in the 200 mg/kg group towards the end of dosing. Males and females in the high dose group exhibited a decrease in blood cell count and hemoglobin, which persisted during the recovery period. Spleen and liver weights were increased in both sexes for the 1000 mg/kg group as well, but only persisted in females during recovery. Blackened spleens were noted in both sexes of the 1000 mg/kg/d group, and was reversible upon recovery. Upon necropsy, a dose-dependent increase in congestion and hemosiderin-laden cells in the spleen and hepatocyte swelling was observed in the 200 mg/kg females, and both sexes in the 1000 mg/kg group. The no-observed-adverse-effect-level (NOAEL) was determined to be 40 mg/kg bw/d, under the conditions of this study.

Tris(Tetramethylhydroxypiperidinol) Citrate

In accordance with OECD TG 407, groups of 5 male and 5 female Sprague-Dawley rats were exposed to 0 (vehicle; water), 100 (low), 500 (mid), or 1000 (high-dose) mg/kg bw/d Tris(Tetramethylhydroxypiperidinol) Citrate in deionized water via gavage for 28 d , and then killed.[4] Two additional groups of 5 males and 5 females were dosed with 0 or 1000 mg/kg/d for 28 d, and then observed post-exposure for 14 d, serving as recovery groups. No mortality occurred during the study. Dose-dependent abnormalities, such as salivation and apparent blood around the facial area, neck and forelimbs, were identified in the males and females dosed with 500 and 1000 mg/kg bw/d. Clinical pathology findings showed a slight increase of serum bilirubin in high-dose male rats, and a statistically significant slight decrease in red blood cell counts (except in mid-dose animals), hemoglobin, and hematocrit in females. Spleen weights were increased in the mid- and high-dose male rats, and there was a minimal to mild increase in the congestion of red pulp of the spleen in several of the male and female rats of the high-dose group. These effects were reversible during the recovery period. The no-observed-effect-level (NOEL) was determined to be 100 mg/kg bw/d.

Subchronic Toxicity Studies

Dermal

Tris(Tetramethylhydroxypiperidinol) Citrate

The dermal toxicity of Tris(Tetramethylhydroxypiperidinol) Citrate (97.3% pure) was evaluated in a 90-d study in Wistar Han rats, in accordance with OECD TG 411.[4] The test substance was administered as a suspension in 0.5% carboxymethylcellulose aqueous solution, and open applications of 0, 50, 150, or 500 mg/kg bw/d were made to the clipped skin of groups of 10 male and 10 female rats. The coverage area was approximately 10% of body surface area (i.e., 45 – 50 cm^2 in males and 30 – 35 cm^2 in females). The animals were killed at the termination of dosing. An additional two groups of 5 males and 5 females received open applications of 0 or 500 mg/kg bw/d of the test substance for 13 wk, and were observed for 4 wk post-dosing as recovery animals. The application sites were not wiped after dosing, and were only cleaned in the instance of excess residue with purified water; ingestion was not prevented. There were no premature deaths. Scabs were noted at the application site during dosing in 2/15 males and 3/15 females dosed with 500 mg/kg bw/d and 1/10 females in both the 50 and 150 mg/kg bw/d. Chorioretinopathy, noted in 2 males and 1 female dosed with 500 mg/kg bw/d, was considered age- and strain-related, and not a test article-related adverse effect. Aberrations in glucose, urea, and potassium concentrations and white blood cell count were also observed in animals given 50 and 500 mg/kg bw/d. The effect on glucose and urea were reversible; however, the effects on white blood cell count and potassium concentrations persisted. An increase in spleen weight and congestion was observed in males and females, but similar congestion was observed in the controls, and the increased weight was reversed in the 500 mg/kg bw/d group of animals following the recovery period. Minimal acanthosis of the epidermis occurred in males and females across all dosing groups, however, it was considered negligible due to similarities in controls. Based on the results of this study, the NOAEL for cutaneous application of the test substance was determined to be 150 mg/kg bw/d.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

Hydroxy Tetramethylpiperidine Oxide

In accordance with OECD TG 414, groups of 22 female Wistar rats were used to evaluate the effects of Hydroxy Tetramethylpiperidine Oxide upon maternal toxicity, as well as embryonic and fetal development.[3] Mated dams were dosed from day 6 to 21 of gestation, via gavage, with 0, 40, 125, or 400 mg/kg bw/d of 98.4 % Hydroxy Tetramethylpiperidine Oxide, in polyethylene glycol. Body weight, appearance and behavioral changes were determined daily during pregnancy, and dams were killed on day 21 of gestation. Mouth rubbing, salivation, and paddling, observed upon immediate administration, and stained fur and minimal sores were considered incidental and not related to the test substance. No adverse effects on maternal reproductive parameters, body weight, food consumption, and post-mortem findings were observed. Several statistically significant changes were observed in the 400 mg/kg dams, including increases in hemoglobin, red blood cell, and reticulocyte count, accompanied by relative increases in spleen weights and aspartate and alanine aminotransferase activity. Kidney dilation, noted in litters from all groups, was statistically significant in the litters of the 400 mg/kg dams; however, in the absence of a dose-response relationship, was not considered toxicologically significant. The NOAEL was determined to be 125 mg/kg/d for maternal toxicity, and 400 mg/kg/d for fetal toxicity.

GENOTOXICITY STUDIES

Details of the genotoxicity studies summarized below are described in Table 4.

In a bacterial reverse mutation assay, Hydroxy Tetramethylpiperidine Oxide was weakly mutagenic when tested at up to 5000 µg/plate in *Salmonella typhimurium* strains TA 100 and 1537, in the presence of metabolic activation.[3] In an Ames test, Tris(Tetramethylhydroxypiperidinol) Citrate did not cause an increase in the mean number of revertants per plate in strains of *S. typhimurium* and *Escherichia coli* WP2 uvr A, when tested at up to 5000 µg/plate, either in the presence or absence of metabolic activation.[4] In a chromosomal aberration test of Tris(Tetramethylhydroxypiperidinol) Citrate tested at up to 5000 µg/plate in Chinese hamster ovary (CHO) cells, there was a weak increase of cell aberrations at the highest dose, in the non-activation assay, and the test substance was not considered genotoxic.[4]

In vivo micronucleus tests were performed with mice. No genotoxicity was observed with 1200 mg/kg bw Hydroxy Tetramethylpiperidine Oxide (administered by gavage),[3] or with up to 200 mg/kg Tris(Tetramethylhydroxypiperidinol) Citrate (administered intravenously).[4]

CARCINOGENICITY STUDIES

No carcinogenicity studies were found in the published literature, and unpublished data were not submitted.

DERMAL IRRITATION AND SENSITIZATION

The dermal irritation and sensitization studies summarized below are described in Table 5.

Hydroxy Tetramethylpiperidine Oxide, at a dose of 0.5 g, did not cause dermal irritation when applied semi-occlusively to male Klein Weisse Russen rabbits, for 4 h, and did not cause sensitization in Pirbright Dunkin-Hartley guinea pigs, tested at the same dose, in a Buehler test.[3] Tris(Tetramethylhydroxypiperidinol) Citrate was deemed non-sensitizing when applied to male and female New Zealand white rabbits for 4 h at a dose of 0.5 g using a semi-occlusive patch, and, when tested at 5.0% w/v in male and female Hartley albino guinea pigs in a maximization test.[4]

In a modified Draize test, up to 0.5% Tris(Tetramethylhydroxypiperidinol) Citrate was dermally tested in 104 human subjects. Adverse events were considered unrelated, and the test substance was deemed non-sensitizing.[4]

OCULAR IRRITATION STUDIES

Animal

Hydroxy Tetramethylpiperidine Oxide

The ocular irritation potential of Hydroxy Tetramethylpiperidine Oxide was evaluated in the eyes of 3 male Klein Weisse Russen rabbits, in accordance with OECD TG 405.[3] An undiluted dose of 0.1 g Hydroxy Tetramethylpiperidine Oxide was instilled into the eye (control not used) for 24 h, after which it was washed with saline. The treated eyes were scored after 24, 48, and 72 h of exposure. Due to an average conjunctiva score of 2.67 (out of 3 max score), average chemosis score of 2 (out of a 4-max score), and 5 of the 12 scored reactions being irreversible, the test material was deemed a Category 1 substance, causing serious and irreversible damage to the eye.

Tris(Tetramethylhydroxypiperidinol) Citrate

The ocular irritation potential of Tris(Tetramethylhydroxypiperidinol) Citrate (93.64% pure) was evaluated in the eyes of 3 female New Zealand White rabbits, in accordance to OECD TG 405.[4] Each rabbit received a 0.027 g (0.1 ml weight equivalent) dose of the undiluted test article, instilled into the conjunctival sac of the right eye, while the other eye remained untreated and served as the corresponding control for each animal. Test and control eyes were examined for signs of irritation for up to 10 d following dosing. The mean irritation score was 0.78 (maximum score of 3), and irritation was fully reversible 72 h to 10 d after exposure. Based on EC Regulation No 1272/2008 (CLP) criteria, the test item was considered non-irritating to rabbit eyes.

SUMMARY

According to the *Dictionary*, Hydroxy Tetramethylpiperidine Oxide is reported to function as an antioxidant, while Tris(Tetramethylhydroxypiperidinol) Citrate is reported to function as a light stabilizer, in cosmetics. In 2020, VCRP data were not available for Hydroxy Tetramethylpiperidine Oxide; Tris(Tetramethylhydroxypiperidinol) Citrate was reported to be used in 388 formulations. According to Council survey data, Hydroxy Tetramethylpiperidine Oxide is reported to be used at a maximum concentration of 12.5% in manicuring preparations (2020), and Tris(Tetramethylhydroxypiperidinol) Citrate at 0.05%, with the highest reported concentration of use reported for cologne and toilet waters and in bath soaps and detergents (2018).

The dermal LD_{50} of Hydroxy Tetramethylpiperidine Oxide was determined to be > 2000 mg/kg bw, in 10 Sprague-Dawley rats exposed to an occlusive patch of 2000 mg/kg bw for 24 h. In an acute dermal toxicity study, 10 New Zealand white rabbits were exposed to an occlusive patch of up to 2136 mg/kg bw of Tris(Tetramethylhydroxypiperidinol) Citrate for 24 h. The dermal LD_{50} was determined to be > 2136 mg/kg bw.

In an acute oral toxicity study, groups of 5 Tif/RAIf rats received 500, 1000, 2000, or 5000 mg/kg bw Hydroxy Tetramethylpiperidine Oxide, via gavage. No mortality occurred in the 500 mg/kg group. Three males and 1 female died in the 1000 mg/kg group, while all males and females died in the 2000 mg/kg and 5000 mg/kg groups. The oral LD_{50} for both sexes (combined) was determined to be 1053 mg/kg bw. In an acute oral toxicity study, groups of 5 Sprague-Dawley rats received up to 3204 mg/kg bw (highest male dose) and 2136 mg/kg bw (highest female dose) of Tris(Tetramethylhydroxypiperidinol) Citrate, by gavage. Three males and 2 females that received the highest dose died prior to scheduled necropsy. The oral LD_{50} for both sexes (combined) was determined to be 1758 mg/kg bw.

In an acute inhalation toxicity, 10 Sprague-Dawley rats were exposed to aerosolized 94.8% pure Tris(Tetramethyl-hydroxypiperidinol) Citrate (estimated MMAD 3.8 µm), at a concentration of 5.08 mg/L, nose-only, for 4 h. The acute inhalation LC_{50} was determined to be greater than 5.08 mg/L.

In an oral study, groups of 6 male and 6 female Sprague-Dawley rats received 0, 8, 40, 200, or 1000 mg/kg bw/d Hydroxy Tetramethylpiperidine Oxide via gavage for 28 d. Two additional recovery groups, consisting of 6 males and 6 females that were administered either the vehicle or the highest dose, were kept alive and observed for 14 d after treatment. Among sacrificed rats, the 1000 mg/kg group had decreased blood cell count and hemoglobin, increased spleen and liver weights, and blackened spleen was observed. A dose-dependent increase in congestion and hemosiderin-laden cells in the spleen and hepatocyte swelling was observed in the 200 mg/kg females, and both sexes in the 1000 mg/kg group. The NOAEL was determined to be 40 mg/kg bw/d. In another 28-d oral toxicity study, groups of 5 male and 5 female Sprague-Dawley rats received up to 1000 mg/kg bw of Tris(Tetramethylhydroxypiperidinol) Citrate via gavage. Two additional groups of 5 males and 5 females were dosed with 0 or 1000 mg/kg/d for 28 d, and then observed post-exposure for 14 d, serving as recovery groups. In the rats that were sacrificed, dose-dependent abnormalities, such as salivation and apparent blood around the facial area, neck and forelimbs, were identified in the males and females dosed with 500 and 1000 mg/kg bw/d; a statistically significant, slight decrease in red blood cell counts, hemoglobin, and hematocrit was seen in females. Spleen weights and congestion also increased, but these effects were reversible during the recovery period. The NOEL was determined to be 100 mg/kg bw/d.

In a 90-d dermal toxicity study, groups of 10 male and 10 female Wistar Han rats were exposed to an open application of up to 500 mg/kg bw/d, 97.3% pure, Tris(Tetramethylhydroxypiperidinol) Citrate. An additional two groups of 5 males and 5 females received open applications of 0 or 500 mg/kg bw/d of the test substance for 13 wk, and were observed for 4 wk post-dosing as recovery animals; no premature deaths occurred. Scabs were noted at the application site during the treatment; aberrations in glucose, urea, white blood cell count, and potassium concentration were also observed in animals in the 50 and 500 mg/kg groups. The effect on glucose and urea was reversible; however, the effects on white blood cell count and potassium concentrations persisted. Based on the results of this study, the NOAEL was determined to be 150 mg/kg bw/d.

In a developmental toxicity study, groups of 22 female Wistar rats were mated, and dosed with up to 400 mg/kg bw/d of 98.4% Hydroxy Tetramethylpiperidine Oxide, via gavage, from day 6 to 21 of gestation. Statistically significant increases in hemoglobin, red blood cell, and reticulocyte count, accompanied by relative increases in spleen weights, aspartate, and alanine aminotransferase activity were observed in the 400 mg/kg dams. In the absence of a dose-response relationship, kidney

dilation in pups from the 400 mg/kg litters was not considered toxicologically significant. The maternal NOAEL was determined to be 125 mg/kg/d, while the fetal NOAEL was determined to be 400 mg/kg/d.

Hydroxy Tetramethylpiperidine Oxide was weakly mutagenic in a bacterial reverse mutation assay, tested at up to 5000 µg/plate. Tris(Tetramethylhydroxypiperidinol) Citrate was not mutagenic in the Ames test, or in a chromosomal aberration assay using CHO cells, when tested at doses up to 5000 µg/plate. In micronucleus assays performed with mice, Hydroxy Tetramethylpiperidine Oxide (1200 mg/kg bw via gavage) and Tris(Tetramethylhydroxypiperidinol) Citrate (up to 200 mg/kg bw, administered intravenously) were not clastogenic.

Hydroxy Tetramethylpiperidine Oxide, at a dose of 0.5 g, did not cause dermal irritation when applied semi-occlusively to Klein Weisse rabbits for 4 h, or when tested at the same dose in a Buehler test, using Pirbright Dunkin-Hartley guinea pigs. Tris(Tetramethylhydroxypiperidinol) Citrate was considered non-sensitizing to the skin of 6 New Zealand White rabbits following semi-occlusive application to a 1 in^2 patch of shaved skin for 4 h. Tris(Tetramethylhydroxypiperidinol) Citrate was not considered a sensitizer in a guinea pig maximization test. In clinical testing with 104 subjects, Tris(Tetramethylhydroxy-piperidinol) Citrate was not a sensitizer.

Hydroxy Tetramethylpiperidine Oxide caused irreversible eye damage when instilled in the eyes of rabbits, at an undiluted dose of 0.1 g. Tris(Tetramethylhydroxypiperidinol) Citrate was considered non-irritating to 3 New Zealand White rabbit eyes. The mean irritation score was 0.78 (maximum score of 3), and irritation was fully reversible between 72 h and 10 d.

DISCUSSION

Hydroxy Tetramethylpiperidine Oxide and Tris(Tetramethylhydroxypiperidinol) Citrate are structurally related as piperidine nitroxides. Therefore, these cosmetic ingredients have been reviewed together in this assessment. Data for a few toxicological endpoints were either not available, or minimal, for the ingredient Tris(Tetramethylhydroxypiperidinol) Citrate. The Panel considered that Hydroxy Tetramethylpiperidine Oxide and Tris(Tetramethylhydroxypiperidinol) Citrate are essentially the same compound, in oxide and citrate salt forms, respectively. Therefore, the Panel felt that data on Hydroxy Tetramethylpiperidine Oxide could be used to substantiate the safety of both ingredients.

Although Tris(Tetramethylhydroxypiperidinol) Citrate is reported to function as a light stabilizer, the Panel discussed that its chemical structure does not have a chromophore and that it is known to act as a free radical scavenger. Hence, it would not be expected to pose phototoxicity concerns.

Initial concerns about the lack of carcinogenicity data were mitigated by sufficient data supporting a lack of genotoxic potential. Additionally, although the Panel noted very limited information on methods of manufacture and impurities for these ingredients, the description for a general synthesis of Hydroxy Tetramethylpiperidine Oxide and the high purity indicated for Tris(Tetramethylhydroxypiperidinol) Citrate (93.64- 97.3%), in conjunction with the lack of adverse effects in a 90-d dermal toxicity study, in which the NOAEL was 150 mg/kg bw/d, mitigated this concern. The safe dermal toxicity profile demonstrated in this report, in addition to a log K_{ow} value of -0.29, indicating minimal dermal penetration, reassured the Panel of safety.

Tris(Tetramethylhydroxypiperidinol) Citrate is reported to be used in products that could possibly be inhaled. For example, this ingredient is used in colognes and toilet waters at concentrations up to 0.05%. The Panel noted that in aerosol products, 95% – 99% of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or bronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Hydroxy Tetramethylpiperidine Oxide and Tris(Tetramehtylhydroxypiperidinol) Citrate are safe in cosmetics in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Chemical Properties

Property	Value	Reference
Hydroxy Tetramethylpiperidine Oxide		
Physical Form (@ 20 °C and 1013 hPa)	Solid, orange flakes	[3]
Formula Weight (g/mol)	172.24	[15]
Topological Polar Surface Area (Å^2)	24.5 (calculated)	[15]
Density/Specific Gravity (g/cm^3 @ 20 °C)	1.127	
Vapor pressure (Pa @ 20 °C)	0.025	[3]
Melting Point (°C)	70 °C	[3]
Partition coefficient (@ 25 °C) log K_{ow}	0.56 (calculated, QSAR)	[3]
Dissociation constant (pKa @ 20 °C)	5.07	[3]
Surface tension (mg/l, in 1.0 g/l distilled water, @ 20 °C)	65.3	[3]
Water solubility (g/l @ 20 °C)	629.3	[3]
Tris(Tetramethylhydroxypiperidinol) Citrate		
Physical Form (@ 20 °C & 1013 hPa)	Solid	[4]
Formula Weight (g/mol)	711.9	[16]
Topological Polar Surface Area (Å^2)	263 (calculated)	[16]
Density/Specific Gravity (g/ml @ 24 ºC)	1.190	[4]
Vapor pressure (Pa @ 20ºC)	< 0.6	[4]
Melting Point (ºC)	59.17 - 64.26	[4]
Boiling Point (ºC)	Decomposed before boiling under nitrogen at atmospheric pressure	[4]
Partition coefficient (@ 20 °C & pH = 4) log K_{ow}	-0.29	[4]
Water Solubility (g/l @ 20.5 ºC)	> 500	[4]

Table 2. Frequency and concentration of use

	# of Uses[6] (2020)	Max Conc of Use (%)[7] (2020)	# of Uses[6] (2020)	Max Conc of Use (%)[8] (2018)
	Hydroxy Tetramethylpiperidine Oxide		Tris(Tetramethylhydroxypiperidinol) Citrate	
Totals*	**NR**	**0.005-12.5**	**388**	**0.0001-0.05**
Duration of Use				
Leave-On	*NR*	*0.005-12.5*	*335*	*0.0001-0.05*
Rinse-Off	*NR*	*NR*	*44*	*0.005-0.05*
Diluted for (Bath) Use	*NR*	*NR*	*9*	*NR*
Exposure Type				
Eye Area	NR	NR	5	0.005
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	154; 138[a]; 26[b]	0.0001-0.05; 0.0001-0.01[a]
Incidental Inhalation-Powder	NR	NR	26[b]; 1[c]	0.005-0.01[c]
Dermal Contact	NR	NR	372	0.0001-0.05
Deodorant (underarm)	NR	NR	2[a]	Not spray: 0.01
Hair - Non-Coloring	NR	NR	15	0.0001-0.01
Hair-Coloring	NR	NR	1	0.005
Nail	NR	0.005-12.5	NR	NR
Mucous Membrane	NR	NR	31	0.01-0.05
Baby Products	NR	NR	1	NR

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

[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

NR – not reported

Table 3. Acute toxicity studies

Ingredient	Animals	No./ Group	Vehicle	Concentration/Dose/Protocol	LD_{50}/Results	Reference
DERMAL						
Hydroxy Tetramethyl-piperidine Oxide	Sprague-Dawley rats	5/sex	water	OECD TG 402. Animals were dosed with 2000 mg/kg bw Hydroxy Tetramethylpiperidine Oxide, in water, via an occluded, 5x6 cm dressing for 24 h.	LD_{50} > 2000 mg/kg bw	[3]
Tris(Tetramethyl-hydroxypiperidinol) Citrate, 93.64%	New Zealand white rabbits	5/sex	Deionized water	OECD TG 402. Limit test involved applying test substance, neat, to 10% of the body surface area. An occlusive application of the substance in deionized water (1 mL of deionized water/g of test substance) at a dose of 2000 mg/kg bw, or 2136 mg/kg bw, was made for 24 h. The rabbits were observed for mortality and clinical abnormalities 14 d before euthanization.	No mortality or significant pathology observed. LD_{50} > 2136 mg/kg bw	[4]
ORAL						
Hydroxy Tetramethyl-piperidine Oxide	Tif/RAIf rats	5/sex	Distilled water	OECD TG 401. Animals received doses of 500, 1000, 2000 or 5000 mg/kg bw, via gavage. There were no controls in the study; the animals were observed for 14 d.	No mortality occurred in the 500 mg/kg group. Three males and 1 female died in the 1000 mg/kg group, while all males and females died in the 2000 mg/kg and 5000 mg/kg groups. LD_{50} values: 953 mg/kg bw (males) 1155 mg/kg bw (females) 1053 mg/kg bw (combined)	[3]
Tris(Tetramethyl-hydroxypiperidinol) Citrate	Sprague-Dawley rats	5/sex	Deionized water	OECD TG 401. Male rats received doses of 1068, 2136, 2670, and 3204 mg/kg bw, while female rats received doses of 534, 1068, 1602, or 2136 mg/kg bw, via gavage. There were no controls in this study. Animals were observed for mortality or clinical abnormalities for 14 d after exposure.	Mortality occurred in 3 male and 2 female rats given the highest dose. These animals exhibited abnormal digestive and pulmonary pathology. LD_{50} values: 2495 mg/kg bw (male) 1068 -1602 mg/kg bw (female) 1758 mg/kg bw (combined)	[4]
INHALATION						
Tris(Tetramethyl-hydroxypiperidinol) Citrate, 94.8%	Sprague-Dawley rats	5/sex	3.8% water, and 0.6% other	OECD TG 403. Animals were exposed nose-only for 4 h to a fine white powder, composed of the test substance which was aerosolized in a gravimetric chamber at a concentration of 5.08 mg/l. The estimated MMAD was 3.8 µm. The animals were observed for mortality and signs of gross toxicity for 14 d after exposure, and then necropsied.	No mortality or gross abnormalities occurred. LC_{50} > 5.08 mg/l	[4]

Table 4. Genotoxicity studies

Test Article	Concentration/ Dose	Vehicle	Test System	Procedure	Results	Reference
			IN VITRO			
Hydroxy Tetramethylpiperidine Oxide*	Up to 5000 µg/plate; with or without metabolic activation	Water DMSO	*Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537	Bacterial reverse mutation assay, in accordance with OECD TG 471	Weakly mutagenic. The test substance was weakly mutagenic (generally, test concentration not specified) in *S. typhimurium* strains TA 100 and 1537, including base-pair and frameshift mutations, in the presence of metabolic activation.	[3]
Tris(Tetramethylhydroxypiperidinol) Citrate, 93.64%	100, 333, 1000, 3330, or 5000 µg/plate; with or without metabolic activation	DMSO	*S. typhimurium* strains TA 1535, TA 1537, TA 98, TA 100 and *Escherichia coli* WP2 uvr A	Ames, mammalian-microsome reverse mutation assay, in accordance with OECD TG 471	Not genotoxic	[4]
Tris(Tetramethylhydroxypiperidinol) Citrate*	Up to 5000 µg/ml; with or without metabolic activation	Water	Chinese hamster ovary cell line (CHO)	Chromosomal aberration test, in accordance with OECD TG 473	Not genotoxic Weak increase in cells with aberrations was observed at the 5000 µg/ml dose. No significant increase in cells with chromosomal abnormalities, polyploidy, or endoreduplication was observed.	[4]
			IN VIVO			
Hydroxy Tetramethylpiperidine Oxide*	1200 mg/kg bw, via gavage	Saline; cyclophosphamide	5 male and 5 female NMRI mice	Micronucleus assay, in accordance with OECD TG 474	Not genotoxic Clinical symptoms such as hunched posture, sedation, piloerection, and death were observed. Induction of micronuclei did not occur.	[3]
Tris(Tetramethylhydroxypiperidinol) Citrate, 93.64%	50, 100, or 200 mg/kg bw, intravenous injection	Water; cyclophosphamide (positive control, given orally)	Groups of 6 male CD-1 mice	Micronucleus assay, in accordance with OECD TG 474. Five animals from the 50 and 100 mg/kg groups and 5 animals from the positive control group were euthanized about 24 h after dosing for bone marrow extraction. Five animals from the 200 mg/kg and 5 from the vehicle group were euthanized about 24 and 48 h after dosing for bone marrow extraction.	Non-clastogenic. Clinical toxicity was observed in the 200 mg/kg animals and 2 animals from this group died. The test item did not induce a statistically significant increase in the frequency of micronucleated polychromatic erythrocytes.	[4]

*Composition not specified
DMSO – dimethyl sulfoxide

Table 5. Dermal irritation and sensitization studies

Test Article	Concentration/ Dose (Vehicle)	Test Population	Procedure	Results	Reference
			ANIMAL		
Hydroxy Tetramethylpiperidine Oxide*	0.5 g (water)	3 male Klein Weisse Russen rabbits	Acute dermal irritation test, in accordance with OECD TG 404. The test article, in 0.5 cm^3 water, was applied to the shaved backs of the animals in a 6 cm^2, semi-occlusive dressing, for 4 h. The test sites were washed with water after exposure and were observed for up to 72 h.	Non-irritating	[3]
Hydroxy Tetramethylpiperidine Oxide*	0.5 g (at 50% w/w, in petrolatum)	29 Pirbright Dunkin-Hartley guinea pigs	Buehler test, in accordance with OECD TG 406. Three, 6-h, occluded induction applications were made to the shaved backs of the animals on day 0, 7, and 14. The challenge application was made, in the same manner, on day 28, and the test site was evaluated at 30 and 54 h after challenge.	Non-sensitizing	[3]
Tris(Tetramethylhydroxypiperidinol) Citrate; 93.64%	0.5 g (water)	3 male and 3 female New Zealand white rabbits	In accordance with OECD TG 404. The test article was applied for 4 h to 1 in^2 of shaved skin using a semi-occlusive patch. Test sites were washed with deionized water after exposure, dried with gauze, and observed for up to 7 d.	Non-irritating Mean erythema score of 1 (maximum score of 4) and mean edema score of 0 were reported; erythema was completely reversible by day 7. According to EC Regulation No. 1272/2008 criteria, was considered non-irritating.	[4]
Tris(Tetramethylhydroxypiperidinol) Citrate*	5.0% w/v; (deionized water)	10 male and 10 female Hartley albino guinea pigs	Guinea pig maximization test, in accordance with OECD TG 406. Intradermal injections of the test substance in deionized water were injected into the animals, along with FCA, and the test article in FCA. The control group (5 male and 5 female guinea pigs) received the same injections, but without the test article. On day 6, 0.5 ml of 10% w/w sodium lauryl sulfate in petrolatum was spread over the intradermal injection sites of all animals. On day 7, any residual sodium lauryl sulfate was removed, and patches with undiluted test article, or water, were applied to the test animals for 48 h. Challenge applications were made on day 20 using Hilltop chambers, and re-challenge applications were made 8 d later in test and control groups	Non-sensitizing Group mean dermal scores were noted to be similar in test animals compared with the challenge control animals.	[4]
			HUMAN		
Tris(Tetramethylhydroxypiperidinol) Citrate*	0.1% or 0.5%; 0.2 ml (in water)	104 subjects	Modified Draize test. Nine occlusive induction applications were made for 24 h with the test article, over 3 wk. The control was water or 0.1% sodium lauryl sulfate. Test sites were wiped with water after each testing phase. After a rest period of 10 -17 d, a previously unexposed site was challenged with the test substance for 24 h.	Non-sensitizing Three adverse events were reported during the course of the study, but they were not related to the exposure to the test substance. The test substance did not appear to cause sensitization during the 3-wk induction period or during the challenge phase.	[4]

*Composition not specified

DCNB – 1,2-dichloro-4-nitrobenzene

FCA- Freund's Complete Adjuvant

NR -not reported

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