Amended Safety Assessment of 5-Amino-6-Chloro-*o*-Cresol as Used in Cosmetics

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*All interested persons are provided 60 days from the above release date (i.e., **May 15, 2023)** to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a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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ABBREVIATIONS

CIR	Cosmetic Ingredient Review
CO_2	carbon dioxide
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Dictionary; wINCI	web-based *International Cosmetic Ingredient Dictionary and Handbook*
DMSO	dimethyl sulfoxide
EC_3	estimated concentrations for a SI of 3
FDA	Food and Drug Administration
HPLC	high-performance liquid chromatography
LLNA	local lymph node assay
LOAEL	lowest-observable-adverse-effect-level
NMR	nuclear magnetic resonance
NOAEL	no-observable-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dose
SI	stimulation indices
TG	test guideline
US	United States
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 5-Amino-6-Chloro-*o*-Cresol, which is reported to function as a hair dye in cosmetic products. The Panel reviewed the available data to determine the safety of this ingredient. The Panel concluded that 5-Amino-6-Chloro-*o*-Cresol is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

5-Amino-6-Chloro-*o*-Cresol, which according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*) is reported to function in cosmetics as a hair colorant,[1] was previously reviewed by the Expert Panel for Cosmetic Ingredient Safety (Panel) as part of a safety assessment of 6 amino-cresol hair dye ingredients that was published in 2004.[2] At that time, the Panel concluded that "the available data … support the safety of 5-Amino-6-Chloro-*o*-Cresol… for use in oxidative and nonoxidative (semi-permanent) hair dyes." In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 years, and it has been at least 15 years since this assessment has been issued. In June 2022, the Panel determined that this safety assessment should be re-opened for re-evaluation due to several of the other amino-cresol hair dye ingredients that were included in the original 2004 report being banned for use in cosmetics by the European Commission.[3] However, because the Panel determined that data for these amino-cresol hair dye ingredients could not be read-across, rather than including all 6 ingredients in one amended report, re-reviews of each hair dye will now be presented as individual stand-alone reports.

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. Excerpts from the summaries of the previous report on 5-Amino-6-Chloro-*o*-Cresol are disseminated throughout the text of this re-review document, as appropriate, and are identified by *italicized text.* (These data are not included in the tables or the Summary.)

CHEMISTRY

Definition and Structure

According to the *Dictionary*, 5-Amino-6-Chloro-*o*-Cresol (CAS No. 84540-50-1) is the organic compound that conforms to formula in Figure 1.[1] However, the use of regiochemical terms such as "ortho-" (i.e., the "-*o*-" in 5-Amino-6-Chloro-*o*-Cresol) is vague and inappropriate when an aromatic system such as a benzene ring has more than 2 substituents.

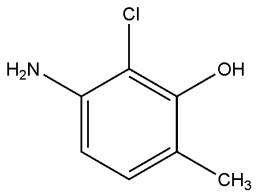

Figure 1. 5-Amino-6-Chloro-*o*-Cresol

5-Amino-6-Chloro-*o*-Cresol is a precursor in oxidative hair dye systems.[4] It reacts with primary intermediates to form the final hair-reactive dye. The reaction can be accelerated by addition of an oxidizing agent (e.g., hydrogen peroxide), but can also be achieved by air oxidation.

Chemical Properties

Chemical properties for 5-Amino-6-Chloro-*o*-Cresol are summarized in Table 1. 5-Amino-6-Chloro-*o*-Cresol is soluble in water and has a symmetrical absorption peak below 300 nm, which falls off sharply above 300 nm.[2]

Method of Manufacture

Method of manufacturing data for 5-Amino-6-Chloro-*o*-Cresol were not included in the original report and were not found in the updated literature search, and unpublished data were not submitted.

Composition and Impurities

A high-performance liquid chromatography (HPLC) analysis of 5-Amino-6-Chloro-o-Cresol yielded 94.19% of the ingredient in one peak.[2] Near the major peak were small peaks for 5-amino-4-chloro-2-methylphenol (2.76%) and p-amino-o-cresol (1.99%). The only other significant peak (0.83%) was identified as a dichloro derivative.

The purity of 5-Amino-6-Chloro-*o*-Cresol is reported to be > 98% (w/w; free base) through nuclear magnetic resonance (NMR) spectroscopy and > 98% (area) through HPLC.[4] Solvent content as water is < 0.5% (w/w). 4-Amino-2-hydroxy-toluene (up to 6%) and 5-amino-4-chloro-*o*-cresol (up to 2.5%) are reported impurities. 5-Amino-4-chloro-o-cresol hydrochloride is a possible impurity.

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics, and does not cover its use in airbrush delivery systems. Data are 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 2022 VCRP survey data, 5-Amino-6-Chloro-*o*-Cresol has 27 reported uses in hair coloring products (Table 2).[5] The results of the concentration of use survey conducted by the Council in 2021 (provided in 2022) reported that the maximum concentration of use of 5-Amino-6-Chloro-*o*-Cresol is 0.24% in hair dyes and colors.[6] When the original safety assessment was published in 2004, 5-Amino-6-Chloro-*o*-Cresol was reported to have no uses (according to VCRP data acquired from the FDA in 1998).[2] However, according to industry survey data submitted in 1996, 5-Amino-6-Chloro-*o*-Cresol was reported to be used at up to 2% in hair dyes and colors.

This ingredient is considered a coal tar hair dye for which regulations require caution statements and instructions regarding patch tests in order to be exempt from certain adulteration and color additive provisions of the US Federal Food, Drug, and Cosmetic Act. In order to be exempt, the following caution statement must be displayed on all coal tar hair dye products:

> Caution - this product contains ingredients which may cause skin irritation on certain individuals and a preliminary test according to accompanying directions should be made. This product must not be used for dyeing the eyelashes or eyebrows; to do so may cause blindness.

Product labels shall also bear patch test instructions for determining whether the product causes skin irritation. However, whether or not patch testing prior to use is appropriate is not universally agreed upon. The Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 h after application of the test material and prior to the use of a hair dye formulation. Conversely, a report in Europe suggests that self-testing has severe limitations, and may even cause morbidity in consumers.[7,8] Hair dye products marketed and sold in the US, though, must follow the labeling requirements established by the Food, Drug, and Cosmetic Act.

In the European Union, 5-Amino-6-Chloro-*o*-Cresol is categorized in Annex III, the list of substances which cosmetic products must not contain except subject to the restrictions laid down.[3] It is limited to 0.5% in non-oxidative hair dyes and to 1% in oxidative hair dyes. The Scientific Committee on Consumer Safety (SCCS) concluded that "because of the low margin of safety for the use in both oxidative and non-oxidative hair dye formulations, … use of 5-Amino-6-Chloro-*o*-Cresol as a hair dye ingredient up to a final on-head concentration of 2.0% under oxidative and non-oxidative conditions poses a risk to the health of the consumer."[4]

TOXICOKINETIC STUDIES

Dermal Absorption/Penetration

In Vitro

The dermal absorption/percutaneous penetration potential of [^{14}C]labelled 5-Amino-6-Chloro-*o*-Cresol (> 99% pure) through dermatomed pig skin was determined for a cream formulation containing 4% of the test material.[4] The material was mixed with a developer and was tested with and without hydrogen peroxide. The study was performed in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 428. The concentration of 5-Amino-6-Chloro-*o*-Cresol in the final application formulation was 2.1%. Using Franz diffusion cells, 20 mg/cm^2 (nominal dose of test substance = 0.42 mg/cm^2; skin discs = 1.0 cm^2) was applied for 30 min. Application of the test material was terminated by gently rinsing with 0.01% Tween 80 solution and water. The formulation was analyzed in 2 experiments with 8 replicates per experiment for adsorbed, absorbed, and penetrated amount of the test material. The receptor fluid (Dulbecco' phosphate buffered saline) was analyzed at defined intervals for up to 48 h post application.

The amount of 5-Amino-6-Chloro-*o*-Cresol systemically available from a standard cream formulation mixed with a developer with hydrogen peroxide was found to be 30.21 ± 9.78 µg/cm^2 (range 18.82 - 49.21 µg/cm^2) or 5.98 ± 1.45% (range 4.24 - 8.22%) of the applied dose. For the formulation tested without hydrogen peroxide, the systemically available amount was 53.8 ± 6.38 µg/cm^2 (range 45.47 - 62.1 µg/cm^2) or 11.78 ± 1.26% (range 9.91 – 13.73%) of the applied dose. The SCCS noted the mean values + 2 standard deviations were used for calculating the margin of safety.[4]

Animal

[^{14}C]5-Amino-6-Chloro-o-Cresol hydrochloride (1.14%) in formulation was readily absorbed (93.2%) in a skin penetration study in female rats.[2] Radioactivity was excreted in urine (87.7%) and feces (2.22%). Only 0.48% was found in the carcass. The recovery rate of ^{14}C from the urine samples was 115% of the applied dose. No detectable radiolabel was found in the expired carbon dioxide (CO_2). In a similar study, the test formulation was mixed 1:1 with 3% hydrogen peroxide developer solution prior to application. The skin penetration was only 0.116%.

Absorption, Distribution, Metabolism, Elimination (ADME) Studies

In Vivo

A metabolism study of [^{14}C]5-Amino-6-Chloro-o-Cresol (0.25% in water) in female rats via a single subcutaneous injection found that excretion was mainly via urine (88.5%), of which most (88.1%) was eliminated in the first 24 h.[2] Only 3.97% was excreted in feces, 0.674% was found in the carcass, and 0.04% remained at the injection site skin. No detectable radioactivity was found in expired CO_2. In an oral metabolism study in male rats, [^{14}C] 5-Amino-6-Chloro-o-Cresol (1.7% in water) was mainly excreted via urine (90.93%), and mostly (90%) in the first 24 h. There was 6% in the gastrointestinal tract and 0.58% in the remaining carcass. No radiolabel was detected in expired CO_2. In a distribution study, male rats received a single oral dose of [^{14}C]5-Amino-6-Chloro-o-Cresol (1.7% in water). After 6 h, radioactivity was in the stomach, intestine, or colon content, and in the cecum. After 24 and 48 h, only residual radioactivity was found in the colon, cecum, and kidneys. After 96 h, excretion was nearly complete and only a small amount of label appeared (in bone). Within the first 24 h, 91% of the radioactivity was excreted via urine.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

The LD_{50} was 1360 mg/kg in a gavage study of male rats that received 5-Amino-6-Chloro-o-Cresol hydrochloride at doses of 501, 1000, 1250, 1580, and 1990 mg/kg.[2] Observations included apathy, staggering, rapid breathing, dyspnea (at later stages), and yellow-orange discoloration of the urine.

Oral

In an acute oral study, 10 male CF1 mice received 501, 631, 794, 1000, 1250, 1580, or 1900 mg/kg bw 5-Amino-6-Chloro-*o*-Cresol dissolved in distilled water by gavage.[9] Clinical symptoms observed in all dose groups included apathy, enhanced breathing, abdominal position, cramps, and uttering of tone. The LD_{50} of the test material was determined to be 1200 mg/kg bw.

Subchronic Toxicity Studies

Oral

In a 13-wk study, male and female rats received daily dose of 50 mg/kg/d 5-Amino-6-Chloro-o-Cresol hydrochloride with tragacanth (1%) by gavage.[2] No clinical observations, biochemical alterations, or pathological findings were indicative of systemic toxicity. The no-observable-adverse-effect-level (NOAEL) was 50 mg/kg.

In a 90-d gavage study, groups of 12 male and 12 female Wistar Crl: (WI) BR rats received 0, 100, 300, or 600 mg/kg/d bw 5-Amino-6-Chloro-*o*-Cresol (> 99.6% pure) in propylene glycol.[4] The study was performed in accordance with OECD TG 408. A recovery group of 5 animals of each sex that received 600 mg/kg bw was also utilized and observed up to 28 d after treatment. Clinical signs of toxicity were monitored daily and feed consumption and body weight gains were measured weekly. Ophthalmologic examinations, hearing tests, and motor tests were also conducted. Blood samples were taken to study hematology, clotting potential, and clinical biochemistry. At necropsy, organ weights were recorded and histopathology of organs was performed.

Three deaths occurred during the study, one in the low-dose group, one in the high-dose group, and one in the recovery control group. All dose groups had a brown discoloration of the urine, and animals in the medium- and high-dose groups were observed with salivation from week 3 of treatment onwards. Histopathology revealed centrilobular hypertrophy of the liver in all groups in a dose-related manner that occurred primarily in males. This finding persisted in all high-dose males through the end of the recovery phase. Other microscopic findings were only related to the high-dose males, including cortical tubular basophilia in the kidneys and limiting ridge hyperplasia of the forestomach with squamous hyperplasia of the main stomach. In the high-dose group (gender not specified), increased relative and absolute liver weights were also observed, along with increased bilirubin, potassium levels, reduced urea levels, increased cholesterol levels, and increased alanine aminotransferase activity. Statistically significant deviations in hematological parameters in the high-dose group included reduced blood cell counts in males, reduced platelet counts in males, increased mean corpuscular volume in males

and females, increased corpuscular hemoglobin level in females, and reduced corpuscular hemoglobin concentration in males and females. In the low-dose group, increased hematocrit values and reduced corpuscular hemoglobin concentration were observed in males, and increased mean corpuscular volume was observed in both males and females. Females in all dose groups were observed to have reduced corpuscular hemoglobin concentrations. Some of these effects persisted through the recovery period. The NOAEL was established at 100 mg/kg/d; however, the SCCS noted that this value should be considered the lowest-observable-adverse-effect-level (LOAEL).[4]

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

No developmental toxicity was associated with treatment with 5-Amino-6-Chloro-o-Cresol hydrochloride in water in rats exposed on days 6 to 15 of pregnancy.[2] The rats received 0, 30, 90, or 270 mg/kg/d of the test material via oral gavage. The dams were killed on day 21 of gestation and the fetuses removed for examination. The only maternal effects were slight reduction in feed consumption and reduced body weight gain in the highest dose group. The NOAEL was considered to be 90 mg/kg/day.

Development and reproductive toxicity studies were not found in the updated literature search, and unpublished data were not submitted.

GENOTOXICITY STUDIES

In an Ames test, 5-Amino-6-Chloro-o-Cresol hydrochloride was evaluated for mutagenicity using concentrations of 4 to 2500 µ g/plate, with and without metabolic activation.[2] 5-Amino-6-Chloro-o-Cresol hydrochloride was mutagenic with metabolic activation in two Salmonella typhimurium strains. No increases in the number of mutations were observed in cell mutation test at the HGRPT locus in Chinese hamster lung fibroblast V79 cells exposed to 5-Amino-6-Chloro-o-Cresol hydrochloride dissolved in ethanol at up to 300 µg/ml, with or without metabolic activation. 5-Amino-6-Chloro-o-Cresol hydrochloride at concentrations at up to 1100 µg/ml did not induce chromosome aberration in Chinese hamster lung fibroblast V79 cells, with or without metabolic activation. No indications of a dose-related increase in unscheduled DNA synthesis were observed in rat liver hepatocytes exposed to 5-Amino-6-Chloro-o-Cresol hydrochloride at up to 2000 µg/ml.

In an vivo micronucleus test for chromosome mutations, 1200 mg/kg of 5-Amino-6-Chloro-o-Cresol hydrochloride dissolved in water was administered to male and female mice via gavage.[2] Bone marrow was extracted for analysis of 1000 polychromic erythrocytes per animal. The ratio of chromatic/polychromatic erythrocytes was slightly increased, suggesting some toxicity to the bone marrow, but the investigators concluded that 5-Amino-6-Chloro-o-Cresol hydrochloride was not mutagenic in this assay.

In vitro and in vivo genotoxicity studies on 5-Amino-6-Chloro-*o*-Cresol summarized below are detailed in Table 3. 5-Amino-6-Chloro-*o*-Cresol (99.6% pure) was not mutagenic in an Ames test at up to 5000 µg/plate, with and without S9 metabolic activation.[4] 5-Amino-6-Chloro-*o*-Cresol (99.6% pure) was not genotoxic in an L5178 mouse lymphoma cells assay at the *tk* locus at up to 1000 µg/ml without metabolic activation or at up to 18.9 µg/ml with metabolic activation. In another gene mutation test, 5-Amino-6-Chloro-o-Cresol (80 - 90% pure) was not genotoxic in Chinese hamster lung fibroblast V79 cells at the HPRT locus, with or without metabolic activation.[9] In vivo testing found that 5-Amino-6-Chloro-*o*-Cresol (99.9% pure) did not induce micronuclei in mice that received a single intraperitoneal dose of up to 400 mg/kg bw.[4]

CARCINOGENICITY STUDIES

Carcinogenicity studies were not included in the original report and were not found in the updated literature search, and unpublished data were not submitted.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

No signs of primary skin irritation were observed in male hairless mice exposed to 5-Amino-6-Chloro-o-Cresol at 10% in an aqueous formulation following twice daily applications for 5 consecutive days.[2] This formulation was also not a dermal irritant in male rabbits following a 2 h application with an occlusive patch or in an open application on the same test site every 30 s for a total of 60 applications.

The irritation potential of undiluted 5-Amino-6-Chloro-*o*-Cresol (99.6% pure) was assessed in 3 male New Zealand albino rabbits in accordance with OECD TG 404.[4] The test material was applied as a single dose (0.5 g) moistened with 0.7 ml purified water to shaved, intact skin. Semi-occlusive patches (2 cm x 3 cm) were applied and left in place for 4 h. The skin was examined for erythema, eschar formation, and edema at 1, 24, 48, and 72 h after the patches were removed. No reactions were observed. It was concluded that 5-Amino-6-Chloro-*o*-Cresol was not irritating or corrosive.

Sensitization

Animal

In a guinea pig maximization study, induction was done with an injection of 5.0% aqueous solution of 5-Amino-6-Chloro-o-Cresol and two injections of 5.0% aqueous 5-Amino-6-Chloro-o-Cresol diluted 1:1 with Freund's complete adjuvant.[2] *A topical induction was done 1 wk later with a 5% cream of 5-Amino-6-Chloro-o-Cresol in petroleum jelly under an occlusive patch for 48 h. The challenge was done with a 25% cream of the test substance applied under an occlusive patch. One quarter of the test animals had slight erythema 24 h after challenge, but no effects were evident after 48 h. It was concluded that 5-Amino-6-Chloro-o-Cresol is not a sensitizer.*

A local lymph node assay (LLNA) was performed using 5-Amino-6-Chloro-*o*-Cresol (99.6% pure) in accordance with OECD TG 429.[4] Female CBA mice were divided into groups of 6 and received 0, 5, 25, or 50% (2 groups) of the test material in ethanol:water (7:3, v/v) on the ear surface (25 µl) once daily for 3 consecutive days. α-Hexylcinnamaldehyde was used as the positive control. Five days after the first topical application, all animals were injected intravenously with [^{3}H]methyl thymidine and the proliferation of lymphocytes in the draining lymph nodes was measured. The stimulation indices (SI) were calculated to be 1.0, 0.9, 4.0, and 1.0 for the 5%, 25%, the initial 50%, and the additional 50% dose groups, respectively. The estimated concentrations for an SI of 3 (EC_3) was not calculated as there was no clear evidence the test material could elicit an SI $\geq$ 3. The controls yielded expected results. It was concluded that 5-Amino-6-Chloro-*o*-Cresol was not sensitizing when tested at up to 50% in mice.

OCULAR IRRITATION STUDIES

Animal

In an ocular irritation study in accordance with OECD TG 405, 3 male New Zealand rabbits received approximately 0.1 ml (38.3 mg) undiluted 5-Amino-6-Chloro-*o*-Cresol (99.6% pure) in the conjunctival sac of one eye.[4] The eye was rinsed with warm tap water after 24 h. The untreated eye served as the control. Ocular reactions were recorded at 1, 24, 48, and 72 h and 7 d after instillation. Slight redness of the conjunctiva was reported in 3 rabbits within 6 h of instillation, which resolved in all animals by 24 h. Exudate was observed in all rabbits 1 h after instillation, in 3 rabbits after 6 h, and in 1 rabbit until 24 h. Opacity (maximum grade 1) and epithelial damage (maximum 55% of the corneal area) of the cornea were observed. The corneal injury resolved with 24 h in one animal and within 7 d in the other animals. Iridial irritation grade 1 was observed in one animal only 24 h after instillation. The irritation of the conjunctivae consisted of redness and chemosis, which resolved within 7 d in two animals and within 14 d in the third. It was concluded that 5-Amino-6-Chloro-*o*-Cresol was an ocular irritant under the conditions of the experiment.

MARGIN OF SAFETY

The SCCS calculated the margin of safety for 2% 5-Amino-6-Chloro-*o*-Cresol under oxidative conditions to be 68.[4] This calculation is based on the adjusted LOAEL of 33 mg/kg bw/d (from a 90-d oral rat study with an adjustment factor of 3) and a systemic exposure dose (SED) of 0.482 mg/kg bw (skin area surface of 580 cm^2 x absorption through skin of 49.77 µg/cm^2 x 0.001 (unit conversion)/typical human bw of 60 kg). The margin of safety for 2% 5-Amino-6-Chloro-*o*-Cresol under non-oxidative conditions was calculated to be 51. In this calculation, the SED value of 0.643 mg/kg bw was used. (The absorption through skin was 66.6 µg/cm^2.)

HAIR DYE EPIDEMIOLOGY

Hair dyes may be broadly grouped into oxidative (permanent) and direct (temporary or semi-permanent) dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes consist of preformed colors. 5-Amino-6-Chloro-*o*-Cresol is reported to be used in direct and oxidative hair dye formulations. While the safety of individual hair dye ingredients is not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer. A detailed summary of the available hair dye epidemiology data is available at https://www.cir-safety.org/cir-findings.

SUMMARY

5-Amino-6-Chloro-*o*-Cresol is reported to function in cosmetics as a hair colorant. 5-Amino-6-Chloro-*o*-Cresol was previously reviewed by the Panel in a safety assessment of 6 amino-cresol hair dye ingredients that was published in 2004. At that time, the Panel concluded that 5-Amino-6-Chloro-*o*-Cresol was safe for use in oxidative and non-oxidative hair dyes. In accordance with its Procedures, the Panel evaluates the conclusions of previously-issued reports approximately every 15 years, and it has been at least 15 years since this assessment has been issued. In June 2022, the Panel determined that this safety assessment should be re-opened for re-evaluation due to several of the other amino-cresol hair dye ingredients that were included in the original 2004 report being banned for use in cosmetics by the European Commission; it should be noted that 5-Amino-6-Chloro-o-Cresol is not currently banned in the European Union, but it does have restrictions.

According to 2022 VCRP survey data, 5-Amino-6-Chloro-*o*-Cresol has 27 reported uses in hair coloring products. The results of the concentration of use survey conducted by the Council in 2021 reported that the maximum concentration of use of 5-Amino-6-Chloro-*o*-Cresol is 0.24% in hair dyes and colors. When the original safety assessment was published in 2004, 5-Amino-6-Chloro-*o*-Cresol was reported to have no uses, according to 1998 VCRP data. However, according to industry survey data submitted in 1996, 5-Amino-6-Chloro-*o*-Cresol was reported to be used at up to 2% in hair dyes and colors.

Under European regulations for cosmetic ingredients, 5-Amino-6-Chloro-*o*-Cresol is categorized in Annex III, the list of substances which cosmetic products must not contain except subject to the restrictions laid down. It is limited to 0.5% in non-oxidative hair dyes and to 1% in oxidative hair dyes. The SCCS concluded that "because of the low margin of safety for the use in both oxidative and non-oxidative hair dye formulations, … use of 5-Amino-6-Chloro-*o*-Cresol as a hair dye ingredient up to a final on-head concentration of 2.0% under oxidative and non-oxidative conditions poses a risk to the health of the consumer."

In a dermal absorption/percutaneous penetration study, a cream formulation containing [^{14}C]5-Amino-6-Chloro-*o*-Cresol (> 99% pure) with a developer was applied to excised pig skin, with and without hydrogen peroxide, with the final concentration of the radiolabel reported as 2.1%. The amount of 5-Amino-6-Chloro-*o*-Cresol systemically available from a standard cream formulation mixed with a developer with hydrogen peroxide was found to be 30.21 $\pm$ 9.78 µg/cm^2 (range 18.82 - 49.21 µg/cm^2) or 5.98 $\pm$ 1.45% (range 4.24 - 8.22%) of the applied dose. For the formulation tested without hydrogen peroxide, the systemically available amount was 53.8 $\pm$ 6.38 µg/cm^2 (range 45.47 - 62.1 µg/cm^2) or 11.78 $\pm$ 1.26% (range 9.91 – 13.73%) of the applied dose.

In an acute oral study in male mice, the LD_{50} for 5-Amino-6-Chloro-*o*-Cresol was 1200 mg/kg bw. The NOAEL in a 90-d oral study in rats was 100 mg/kg/d. Adverse effects included centrilobular hypertrophy of the liver observed at all dose levels in a dose-dependent manner that persisted through the recovery period. High-dose male group also had cortical tubular basophilia in the kidneys, limiting ridge hyperplasia of the forestomach with squamous hyperplasia of the main stomach, and increased relative and absolute liver weights. A reduced mean corpuscular hemoglobin concentration was observed in all dose groups of female rats.

5-Amino-6-Chloro-*o*-Cresol (99.6% pure) was not mutagenic in an Ames test at up to 5000 µg/plate, with and without S9 metabolic activation. 5-Amino-6-Chloro-*o*-Cresol (99.6% pure) was not genotoxic in a L5178 mouse lymphoma cell assay at the *tk* locus at up to 1000 µg/ml without metabolic activation or at up to 18.9 µg/ml with metabolic activation. In another gene mutation test, 5-Amino-6-Chloro-*o*-Cresol (80-90% pure) was not genotoxic in Chinese hamster lung fibroblast V79 cells at the HPRT locus, with or without metabolic activation. In vivo testing found that 5-Amino-6-Chloro-*o*-Cresol (99.9% pure) did not induce micronuclei in mice that received a single intraperitoneal dose of up to 400 mg/kg bw.

Undiluted 5-Amino-6-Chloro-*o*-Cresol was not irritating or corrosive in a dermal study in rabbits. This ingredient was also not sensitizing in mice in an LLNA when tested at up to 50%. Undiluted 5-Amino-6-Chloro-*o*-Cresol was an ocular irritant in rabbit eyes.

A margin of safety for 2% 5-Amino-6-Chloro-*o*-Cresol under oxidative conditions was calculated to be 68. This calculation is based on the adjusted LOAEL of 33 mg/kg bw/d (from a 90-d oral rat study with an adjustment factor of 3) and an SED of 0.482 mg/kg bw. The margin of safety for 2% 5-Amino-6-Chloro-*o*-Cresol under non-oxidative conditions was calculated to be 51. In this calculation, the SED value of 0.643 mg/kg bw was used.

The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer.

Method of manufacturing data and carcinogenicity studies on 5-Amino-6-Chloro-*o*-Cresol were not included in the original report and were not found in the updated literature search, and unpublished data were not submitted.

DISCUSSION

5-Amino-6-Chloro-*o*-Cresol is reported to function as a semi-permanent and oxidative hair dye in hair coloring products. The Panel has determined that the data are sufficient to support safety of this ingredient in hair dye products, which are rinsed-off after application. The Panel noted that the available data show that 5-Amino-6-Chloro-*o*-Cresol absorbs slowly through the skin, is not genotoxic, and has low concentrations of use. The Panel considered these findings, coupled with the short exposure time as a rinse-off product, and determined that the data are sufficient to conclude that 5-Amino-6-Chloro-*o*-Cresol is safe for use as a hair dye ingredient in the present practices of use and concentration.

The Panel recognizes that hair dyes containing this ingredient, as coal tar hair dye products, are exempt from certain adulteration and color additive provisions of the Federal Food, Drug, and Cosmetic Act (FD&C Act), when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures. The Panel considered concerns that such self-testing might induce sensitization, but agreed that there was not a sufficient basis for changing this advice to consumers at this time.

In considering hair dye epidemiology data, the Panel concluded that the available epidemiology studies are insufficient to scientifically support a causal relationship between hair dye use and cancer or other toxicological endpoints, based on lack of strength of the associations and inconsistency of findings. Use of direct hair dyes, while not the focus in all investigations, appears to have little evidence of any association with adverse events as reported in epidemiology studies.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that 5-Amino-6-Chloro-*o*-Cresol is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Chemical properties

Property	Value	Reference
Physical Form	Beige crystals	[2]
Formula Weight (g/mol) Molecular Weight (g/mol)	194.07 (hydrochloride) 157.6 (free base)	[2] [4]
Melting Point (ºC)	144-183 82-86	[2] [4]
Water Solubility (g/l @ 20 ºC)	< 10	[4]
Other Solubility (g/l @ 20 ºC)	ethanol: < 100 dimethyl sulfoxide (DMSO): > 100	[4]
log P_{ow}	1.644 (experimental) 1.44 (estimated)	[4]

Table 2. Updated and historical frequency (2022; 1998) and concentration (2021; 1996) of use by product category

	# of Uses		*Max Conc of Use (%)*	
	5-Amino-6-Chloro-*o*-Cresol			
	2022[5]	**1998[2]**	**2021[6]**	**1996[2]**
Totals	**27**	**NR**	**0.007 – 0.24**	**2**
Hair Coloring Preparations				
Hair Dyes and Colors (all types requiring caution statements and patch tests)	26	NR	0.24	2
Hair Bleaches	1	NR	0.007	NR

NR – not reported

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Table 3. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
			IN VITRO			
5-Amino-6-Chloro-*o*-Cresol; 99.6% pure	3 - 5000 µg/plate	DMSO	*Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	Bacterial reverse mutation test in accordance with OECD TG 471; with and without S9 metabolic activation	Not mutagenic; no biologically relevant increase in revertant colonies observed in any strain, with or without metabolic activation	[4]
5-Amino-6-Chloro-*o*-Cresol; 99.6% pure	Test 1: up to 1000 µg/ml without metabolic activation and up to 18.9 µg/ml with metabolic activation Test 2: up to 400 µg/ml without metabolic activation and up to 18 µg/ml with metabolic activation	DMSO	L5178 mouse lymphoma cells	Mammalian cell gene mutation test at the *tk* locus in accordance with OECD TG 476; with and without metabolic activation	Not genotoxic; no reproducible increase in mutant frequency observed in both tests without metabolic activation; a dose-related increase in mutant frequency observed only in test 1 with metabolic activation, but increases were not reproducible in test 2 and the effect was considered not biologically relevant	[4]
5-Amino-6-Chloro-*o*-Cresol; 80 - 90% pure	Up to 350 µg/ml without metabolic activation and up to 3000 µg/ml with metabolic activation	DMSO	Chinese hamster fibroblast V79 cells	Mammalian cell gene mutation test at the HPRT locus in accordance with OECD TG 476; with and without metabolic activation	Not genotoxic; no biologically relevant significant increase in mutant colony numbers, with or without metabolic activation; negative and positive controls according to guidelines	[9]
			IN VIVO			
5-Amino-6-Chloro-*o*-Cresol; 99.9% pure	100, 200, and 400 mg/kg bw	DMSO (30%)	5 NMRI mice per sex	Mammalian erythrocyte micronucleus test in accordance with OECD TG 474; single intraperitoneal dose; groups of animals killed at 24 or 48 h post-treatment; appropriate negative and positive controls used	Not genotoxic; test material did not induce micronuclei. No mortalities observed; clinical signs included reduced spontaneous activity, ruffled fur, eyelid closure, and abdominal position	[4]

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