Amended Safety Assessment of 6-Amino-*m*-Cresol as Used in Cosmetics

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All interested persons are provided 60 days from the above release date (i.e., August 26, 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to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to the Cosmetic Ingredient Review (CIR) will be discussed in open meetings, will be available for review by any interested party, and may be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.

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

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ABBREVIATIONS

ADME	absorption, distribution, metabolism, excretion
CIR	Cosmetic Ingredient Review
CMC	carboxymethylcellulose
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
DMSO	dimethyl sulfoxide
DNCB	dinitrochlorobenzene
EC_3	effective concentration inducing a stimulation index of 3
FDA	Food and Drug Administration
LLNA	local lymph node assay
NMR	nuclear magnetic resonance
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
P_{app}	apparent permeability coefficient
SCCS	Scientific Committee on Consumer Safety
TG	test guideline
US	United States
UV	ultraviolet
VCRP	Voluntary Cosmetic Registration Program
wINCI; *Dictionary*	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 6-Amino-*m*-Cresol, which is reported to function as a hair colorant in cosmetic products. The Panel reviewed the available data to determine the safety of this ingredient. The Panel concluded that the available data are insufficient to make a determination of safety for 6-Amino-*m*-Cresol under the intended conditions of use as a hair dye ingredient.

INTRODUCTION

6-Amino-*m*-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 six 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 6-Amino-*m*-Cresol… as used in oxidative and non-oxidative (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 due to 6-Amino-*m*-Cresol being banned for use in cosmetics by the European Commission.[3,4] 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 extensive search of the world's literature; this search was last performed April 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 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 6-Amino-*m*-Cresol are disseminated throughout the text of this re-review document, as appropriate, and are identified by *italicized text*. (This information is not included in the tables or the Summary section.)

CHEMISTRY

Definition and Structure

According to the *Dictionary*, 6-Amino-*m*-Cresol (CAS No. 2835-98-5) is the substituted aromatic compound that conforms to formula in Figure 1.[1] However, the use of regiochemical terms such as "*meta*-" (i.e., the "-*m*-" in 6-Amino-*m*-Cresol) is vague and inappropriate when an aromatic system such as a benzene ring has more than 2 substituents. Thus, a technical name, such as 2-amino-5-methylphenol, is more common in the literature.

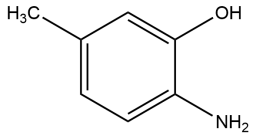

Figure 1. 6-Amino-*m*-Cresol

6-Amino-*m*-Cresol is used as a coupler in oxidative hair dye systems. Couplers, sometimes referred to as color modifiers, react with oxidized hair dye ingredients referred to as precursors. Couplers can react with 2 equivalents of precursor, or if "blocked," react with 1 equivalent of precursor to form multimeric chemical species. The methyl group on 6-Amino-*m*-Cresol, however, does not block coupling at either active site (i.e., neither *ortho*- nor *para*-); thus, this ingredient theoretically can react with precursors to form dimer- or trimer-like products (Figure 2).

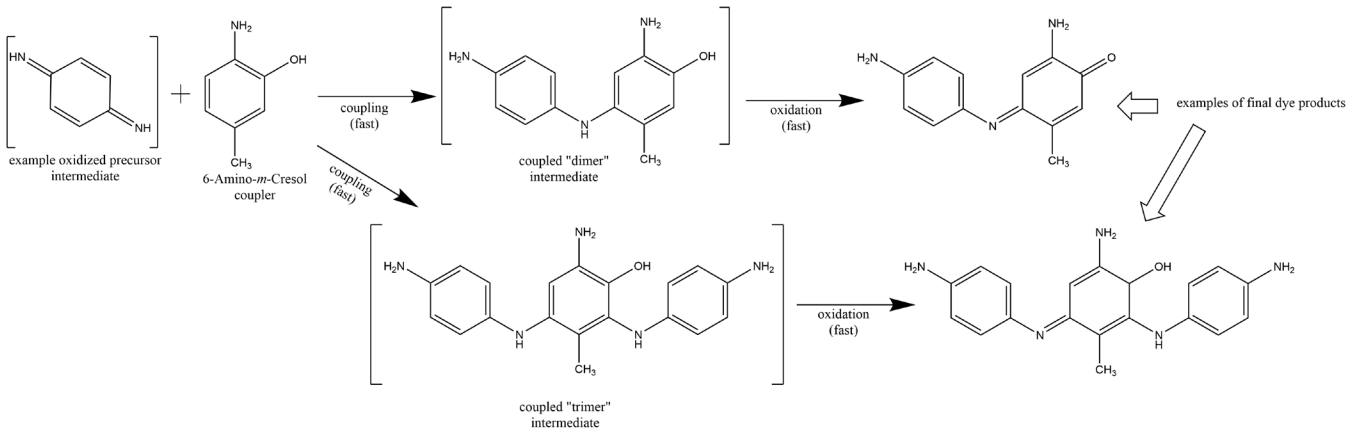

Figure 2. Examples of hair dye coupling with 6-Amino-*m*-Cresol

Chemical Properties

Chemical properties for 6-Amino-*m*-Cresol are summarized in Table 1. 6-Amino-*m*-Cresol (99.9% pure) has a molecular weight of 123.16 g/mol and is in the form of beige to reddish-brown crystals.[2] The estimated log P_{ow} is reported to be 1.14.[4]

Method of Manufacture

Method of manufacturing data were not included in the original report, were not found in the updated literature search, and unpublished data were not submitted.

Constituents/Impurities

Four different batches of 6-Amino-*m*-Cresol analyzed with nuclear magnetic resonance (NMR) were determined to be 98.9% - 99.7% (w/w) pure.[4] Water content ranged from 0.017% - 0.1% (w/w) and sulfated ash content ranged from < 0.01% - < 0.1% (w/w). Other constituents included 2-amino-4-methylphenol (< 1000 ppm, detection limit), 1,2-diamino-4-methylbenzene (< 20 - < 59 ppm, detection limit), and 4-methyl-2-nitroaniline (< 10 - < 23, detection limit).

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 2023 VCRP survey data, 6-Amino-*m*-Cresol has no reported uses.[5] The results of the concentration of use survey provided by the Council in 2022 reported that this ingredient is used at 0.69% in hair dyes and colors, indicating use in at least 1 cosmetic formulation.[6] When the original safety assessment was published in 2004, 6-Amino-*m*-Cresol was reported to have 2 uses in hair dye and color formulations, according to 1998 VCRP data.[2] At that time, 6-Amino-*m*-Cresol was reported to be used at 2.4% in hair dyes and colors according to a survey performed by industry.

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.

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.

Under European regulations for cosmetic ingredients, 6-Amino-*m*-Cresol is categorized in Annex II, the list of substances prohibited in cosmetic products in Europe.[3] The European Union's Scientific Committee on Consumer Safety (SCCS) determined that 6-Amino-*m*-Cresol could not be considered safe for hair dying purposes due to the genotoxic potential of this ingredient and its metabolite, *N*-acetyl-2-amino-5-methylphenol.[4] This determination was coupled with data that indicated that a dimer of the ingredient was formed under oxidative conditions and was found to absorb in human skin (in vitro). The SCCS assessors objected to the INCI name, stating it was scientifically incorrect, and referred to this ingredient as 2-amino-5-methylphenol in their opinion.

TOXICOKINETIC STUDIES

Dermal Absorption

In Vitro

The dermal penetration potential of [^{14}C]6-Amino-*m*-Cresol (99% radiochemical purity) from a typical oxidative hair dye formulation was studied in viable human donor skin in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 428.[4] The formulation tested containing 0.75% [^{14}C]6-Amino-*m*-Cresol (with a reaction partner and hydrogen peroxide) was applied to the skin samples for 60 min. The dose per unit area was 100 mg/cm^2. Absorption was assessed by collecting receptor fluid (Dulbecco's minimum eagle medium) samples at 3 and 24 h post-dosing. At 24 h, the experiment was terminated by removing the skin samples from the well plates. The wells were rinsed with solvent and the skin was dried and the stratum corneum was removed by tape stripping. Exposed skin underwent an extraction procedure. Radioactivity was measured by liquid scintillation counting. The dermal delivery (amount of test material in the receptor fluid and in the skin) of the oxidative hair dye formulation containing 0.75% 6-Amino-*m*-Cresol was determined to be 0.34 % (2.77 µg/cm^2).

Animal

In an in vivo dermal absorption study, 6 male and 6 female PVG rats received [^{14}C]6-Amino-*m*-Cresol hemisulfate (95% radiochemical purity) in dimethyl sulfoxide (DMSO; 150 mg/ml; 0.1 ml/animal for 24 h) and in a formulation (15 mg/g; 1 g mixture/animal for 0.5 h) on the dorso-lumbar region.[4] The test sites were occluded (15 mg/animal; 1.667 mg/cm^2, 190 mCi). At the end of the dose periods, the plaster and foil were removed, and the animals were kept in individual metabolism cages to collect urine, feces, and expired air. The animals were killed 72 h post-doing and tissues were removed for radioactivity analysis. Approximately 14.25% of the applied dose of [^{14}C]6-Amino-*m*-Cresol dissolved in DMSO and 0.58% of the applied dose of the test material in a hair dye formulation were excreted, mainly in the urine. The remaining applied doses of the test material in DMSO (74.48%) and in formulation (82.78%) were recovered from the dressing, washing and application sites. No significant radioactivity levels were found in tissues 72 h after treatment.

Absorption, Distribution, Metabolism, Excretion (ADME)

In Vitro

The bioavailability of 6-Amino-*m*-Cresol (98.8% pure) across the intestinal barrier was investigated in human intestinal epithelial (TC-7) cells in vitro.[4] 6-Amino-*m*-Cresol (96 % recovery) revealed an apparent permeability coefficient (P_{app}) of 129.9 x 10^{-6} cm/sec and thus was classified to be of high permeability, indicating a complete absorption from the gastrointestinal tract. As the absorption from the gastrointestinal tract is likely to be permeability limited, the high permeability observed in this assay indicates a good absorption of 6-Amino-*m*-Cresol after oral administration.

The metabolism of 6-Amino-*m*-Cresol (98.8% pure) was studied in human, rat and mouse primary hepatocytes.[4] The test material (10µM) was incubated with the hepatocytes for 4 h. The metabolism of 6-Amino-*m*-Cresol was similar between the 3 hepatocyte types. The test material was extensively metabolized by sulfate and glucuronide conjugation. Although the human donors were phenotyped as rapid acetylators, no *N*-acetyl-2-amino-5-methylphenol could be detected.

In another metabolism study of [^{14}C]6-Amino-*m*-Cresol (97.9% - 98.9% radiochemical purity), human hepatocytes were suspended with 1, 10, 100, or 1000 µg/ml of the test material for 3 h or plated with 0.889, 8.89, or 88.9 µg/ml of the test material for 24 h.[4] The metabolic activity was assessed by measuring 7-hydroxycoumarin formation. [^{14}C]6-Amino-*m*-Cresol was readily metabolized and the profile of metabolites formed was concentration-dependent. Two metabolites of 6-Amino-*m*-Cresol, *O*-glucurono-2-amino-5-methylphenol and 2-amino-5-methylphenol-*O*-sulfate, were detected in studies with both suspended and plated hepatocytes. The substrate concentration dependency of formation of these metabolites was

similar in both studies. A third metabolite, *N*-acetyl-*O*-glucurono-2-amino-5-methylphenol, was detected in minor amounts only with plated hepatocytes, a test system that may more closely reflect the in vivo metabolic capability. In plated hepatocytes at the low-test concentration, the major metabolite was 2-amino-5-methylphenol-*O*-sulfate, while at higher substrate concentrations, *O*-glucuronidation became the predominant metabolic pathway. Metabolism was incomplete at the high concentrations both in suspended and plated human hepatocytes, suggesting saturation of phase II metabolism or enzyme inhibition.

The metabolism of an oxidative hair dye formulation containing 1.5% [^{14}C]6-Amino-*m*-Cresol (99% radiochemical purity) was investigated in viable human skin (thickness 580 - 650 µm) obtained from 3 female donors.[4] The skin samples (100 mg/cm^2) were exposed for 60 min to the test material. At 24 h post-dosing, the experiment was terminated by removing the skin samples. The samples of the receptor fluid (Dulbecco's minimum eagle medium) and skin extract were analyzed for metabolite profiling and identification. The metabolite profiling results indicate that *N*-acetylation is the major route of metabolism of 6-Amino-*m*-Cresol in skin. *N*-Acetyl-2-amino-5-methylphenol, 2-methyl-5-aminophenol-*O*-sulfate, and N-acetyl -2-amino-5-methylphenol-*O*-sulfate were identified as metabolites. A fourth metabolite (postulated to be *N*-acetyl-*O*-glucurono-2-amino-5-methylphenol) was detected in both receptor fluid and exposed skin samples. A dimer of 6-Amino-*m*-Cresol and a related substance were also identified in the receptor fluid and skin extract samples but were not quantified. The dimer was likely formed under the oxidative conditions of the formulation. The amount of *N*-acetylated metabolites was calculated to be 0.93 µg/cm^2; i.e., at least 34% of the total amount of 6-Amino-*m*-Cresol that was found in the receptor fluid or in the skin (2.77 µg/cm^2) was present in the form of these *N*-acetylated metabolites. However, the acetylator status of the skin samples of the 3 donors regarding arylamine *N*-acetyltransferase 1 (rapid or slow) is unknown. Thus, apart from other methodological restrictions, the evidence on the *N*-acetylation metabolic pathway in human skin is at best of semi-quantitative nature.

Animal

The ADME of [^{14}C]6-Amino-*m*-Cresol (97.9% radiochemical purity) was studied in Sprague-Dawley female rats after a single oral, intravenous, or dermal dose.[4] A total of 8 groups was used, with 4 groups used for a mass balance study and the remaining 4 groups used for toxicokinetics. Groups that received 25 or 400 mg/kg bw test material (in PEG 400) orally were comprised of 6 rats. Groups that received intravenous administrations of 25 mg/kg bw of the test material (in PEG 400/0.9% saline 40:60) were comprised of 4 rats. The groups that received 10 mg/kg bw test material (in acetone/ water 1:1) dermally on shaved skin (10 cm^2) were comprised of either 4 or 6 rats. In the mass balance groups, urine and feces were collected in intervals of 0 - 8, 8 - 24, 24 - 48, 48 - 72, and 72 - 96 h. Total radioactivity in urine, feces, tissues, and organs was determined. Selected urine and feces samples were pooled per group and the metabolite profile was investigated. In the toxicokinetics groups, blood was sampled alternatively from several rats at 0.25, 0.5, 1, 2, 4, 8, 24, and 48 h after dosing.

[^{14}C]6-Amino-*m*-Cresol administered orally was well absorbed, readily distributed, extensively metabolized and excreted mainly via urine. There is weak analytical evidence that metabolism resulted in oxidized and *N*-acetylated derivatives. After dermal application, 5.1% (0.019 mg/cm^2) of the radiolabeled dose was found in excretion, cage-wash, carcass and unexposed skin. This amount increased to 6.8% (0.026 mg/cm^2) when adding the residue in the exposed skin. Excretion took place mainly via urine, but elimination was slower compared to oral administration. Intravenous results were not reported.[4]

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

The LD_{50} was calculated to be 1500 mg/kg in an acute study of male CD-1 mice dosed for 2 consecutive days with up to 1500 mg/kg 6-Amino-m-Cresol.[2] In a pre-experiment toxicity study, the maximum tolerated dose was 500 mg/kg in NMRI mice that received up to1500 mg/kg 6-Amino-m-Cresol in PEG 400. Mice that received 666 mg/kg 6-Amino-m-Cresol orally had tremor, anemia, and a slight to moderate reduction in activity within the first 2 h of being treated; however, no mortalities were observed. Two male rats that were orally dosed with 1200 mg/kg 6-Amino-m-Cresol in 1% carboxymethylcellulose had reduction of spontaneous activity, abdominal position, eyelid closure, and piloerection. In another experiment, the maximum tolerated dose was 1500 mg/kg in male rats that received either a single oral dose of 1500 or 2000 mg/kg 6-Amino-m-Cresol in 1% carboxymethylcellulose.

In an oral acute toxicity study, 6 male and 10 female Wistar rats received up to 1750 mg/kg bw 6-Amino-*m*-Cresol (purity not reported) via oral gavage, while 10 male and 10 female CF1 mice received up to 2000 mg/kg bw of the test material and 10 female CBL mice received up to 1250 mg/kg bw.[4] Mortality and clinical signs were checked daily during the 14-d observation period. All animals underwent gross necropsy after termination. Clinical signs of toxicity observed included sedation, tremor, accelerated respiration, and death. No macroscopic organ changes were noted. The LD_{50} were calculated as follows: 1375 mg/kg bw in male rats, 1225 mg/kg bw in female rats, 1020 mg/kg bw in male CF1 mice, 1225 mg/kg bw in female CF1 mice, and 750 mg/kg bw in CBL female mice.

Short-Term Toxicity Studies

Oral

Male and female Wistar rats (15/sex/group) were dosed orally with 50, 250, and 500 mg/kg 6-Amino-m-Cresol daily for 4 wk.[2] The control group was dosed with 1 ml/100 g bw 0.5% carboxymethylcellulose (CMC). No significant observations occurred in the 50 mg/kg group. The 250 mg/kg group had increased activity during the third and fourth week of treatment and increased, discolored urine excretion. Significant results included reduced erythrocyte counts in males (highly significant) and females; increased reticulocytes in females; decreased hemoglobin in males and a highly significant decrease in females; increased hematocrit in both sexes, but highly significant in males; decreased iron in females; increased hepatic weight in females; increased kidney weight in males and females; and increased spleen weights in both sexes, but highly significant in females. The 500 mg/kg group had initial decreased activity during week 1 and later, increased activity as in the previous group. Increased, discolored urine excretion was also observed. Borderline significant results were observed for decreased body weight gain and feed consumption during weeks 1 and 2 in females. Highly significant results were reported for increased water consumption in both sexes at all phases of the study; decreased erythrocytes and hemoglobin and increased reticulocytes in both sexes; and decreased hematocrit in males and females, although females were within normal range. The mean corpuscular volume and prothrombin time was significantly increased in females, but still in the normal range. At necropsy, dark, discolored spleens were observed (sex not specified). Liver, kidney, and spleen weights were all increased in both sexes. No treatment related observations were observed at microscopic evaluation. The no-observed-adverse-effect level (NOAEL) for 6-Amino-m-Cresol was established at 50 mg/kg.

Subchronic Toxicity Studies

Oral

In a 90-d oral gavage study, 10 male and 10 female Wistar Bor: WISW/TNO (SPF) rats received a 10% suspension of 6-Amino-*m*-Cresol (98% pure) in 5% gum arabic at a dose of 800 mg/kg bw/d.[4] After week 6, the dose was reduced to 500 mg/kg bw/d due to clinical signs of toxicity and 2 animal deaths. Feed consumption, body weight, and body weight gains were significantly reduced in both sexes. Relative and absolute liver, kidney, and spleen weights were increased. No macroscopic or histopathological effects detected. An NOAEL could not be calculated.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

Female Sprague-Dawley rats dosed orally with 5, 50, or 200 mg/kg 6-Amino-m-Cresol from days 6 to 15 of gestation had no mortalities attributed to treatment effects.[2] When compared to controls, no clinical changes or changes at necropsy were observed in any group. Body weight gain of all treated groups was comparable to the control group. No effect on pregnancy incidence was observed in the treated groups. The positive control group had marked teratogenic effects: the majority of fetuses had exencephaly. 6-Amino-m-Cresol did not elicit embryotoxicity, embryolethality, or teratogenicity.

GENOTOXICITY STUDIES

In an Ames test using Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100, 6-Amino-m-Cresol (30 - 1000 µg/plate, with or without equal amounts of 6% hydrogen peroxide) was slightly mutagenic towards strain TA100 with and without metabolic activation.[2] It was not mutagenic towards the other strains. 6-Amino-m-Cresol (0.6 - 15.0 µg/ml in DMSO) was highly toxic to Saccharomyces cerevisiae diploid D7 cell cultures, with and without metabolic activation, but it did not induce increases in the frequency of convertant or aberrant colonies. 6-Amino-m-Cresol (12.5 - 200 µg/ml in DMSO) induced an increase in mutation to both ouabain and 6-thioguanine resistance in mouse lymphoma L5178Y cells with metabolic activation; however, the increase was not considered significant with or without metabolic activation. In a study using cultured male human peripheral lymphocytes, 6-Amino-m-Cresol hemisulfate (0.6 - 15.0 µg.ml in DMSO) did not significantly increase the number of aberrations as compared to controls, with or without metabolic activation.

In in vivo micronucleus tests in mice, 6-Amino-m-Cresol (up to 750 mg/kg) did not induce micronuclei in bone marrow cells.[2] 6-Amino-m-Cresol (3200 mg/kg in 4% gum Arabic) did not induce chromosome aberrations in Chinese hamster bone marrow cells. A cytotoxic effect was observed, which indicated a strongly decreased ratio of polychromatic and normochromatic erythrocytes in the bone marrow (55% reduction compared to control animals). 6-Amino-m-Cresol hemisulfate did not cause sister chromatid exchanges in rat bone marrow chromosomes from a single oral dose of up to 600 mg/kg in distilled water. 6-Amino-m-Cresol did not induce unscheduled DNA synthesis assay at up to 1500 mg/kg. One of the animals in the 1500 mg/kg dose group died within 16 h of treatment, and the other animals in the group had signs of toxicity. Additionally, the hepatocyte viability of two animals out of the 1500 mg/kg group was decreased.

In vivo and in vitro genotoxicity studies for 6-Amino-*m*-Cresol are summarized in Table 2. 6-Amino-*m*-Cresol (0 - 5000 µg/plate in DMSO; 98.8% pure) was mutagenic in *S. typhimurium* strain TA100, with and without metabolic activation, but no mutagenicity was observed in strains TA98, TA102, TA1535, or TA1537.[4] Clastogenic effects were observed in a concentration-dependent and biologically relevant manner in a mouse lymphoma L5178Y $tk^{+/-}$ cell gene mutation test of 6-Amino-*m*-Cresol (97.8% pure) at up to 100 µg/ml with metabolic activation. A biologically relevant increase in mutant

frequency was not observed at up to 160 µg/ml without metabolic activation. 6-Amino-*m*-Cresol (98.8% pure) induced an increase in human lymphocytes with micronuclei when tested at up to 26.8 µg/ml without metabolic activation, but no genotoxicity was observed when tested at up to 67.7 µg/ml with metabolic activation. In an alkaline Comet assay, a concentration-dependent and biologically relevant increase in the amount of DNA in the tail was observed when 6-Amino-*m*-Cresol (98.8% pure) was tested at up to 1232 µg/ml, with and without metabolic activation. In a rat micronucleus test, 6-Amino-*m*-Cresol (single intraperitoneal injection of up to 400 m/kg bw; purity not reported) induced a biologically relevant and dose-dependent increase in the number of bone marrow cells with micronuclei in both sexes.

CARCINOGENICITY STUDIES

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

DERMAL IRRITATION AND SENSITIZATION STUDIES

Dermal irritation and sensitization studies for 6-Amino-*m*-Cresol are summarized in Table 3. 6-Amino-*m*-Cresol (purity not reported) was not irritating in guinea pigs when tested at 1% in water and thickened with methylcellulose.[4] In a guinea pig sensitization test, no sensitization was observed to 3% 6-Amino-*m*-Cresol (purity not reported) in water and thickened with tylose. In a local lymph node assay (LLNA), 6-Amino-*m*-Cresol (98.9% pure) was a strong skin sensitizer when tested at up to 10% in DMSO (effective concentration inducing a stimulation index of 3 (EC_3) of 3.44%) and at up to 5% in acetone: water (1:1) mixed with olive oil (3:1) (EC_3 of 1.55%).

OCULAR IRRITATION STUDIES

In an ocular irritation study, 10 female Pirbright SPF guinea pigs had 1% 6-Amino-*m*-Cresol (aq.; purity not reported) instilled into 1 eye (0.1 ml).[4] No irritation was observed after the 24 h observation period. No further details provided.

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. 6-Amino-*m*-Cresol is reported to be used in semi-permanent 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

6-Amino-*m*-Cresol is reported to function in cosmetics as a hair colorant. 6-Amino-*m*-Cresol was previously reviewed by the Panel in a safety assessment that was published in 2004. At that time, the Panel concluded that 6-Amino-*m*-Cresol was safe as used in oxidative and non-oxidative (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 due to 6-Amino-*m*-Cresol being banned for use in cosmetics by the European Commission.

No uses were reported for 6-Amino-*m*-Cresol, according to 2023 VCRP data. The results of the concentration of use survey provided by the Council in 2022 report that this ingredient is used at 0.69% in hair dyes and colors, indicating use in at least 1 cosmetic formulation. When the original safety assessment was published in 2004, 6-Amino-*m*-Cresol was reported to have 2 uses in hair dye and color formulations, according to 1998 VCRP data. At that time, 6-Amino-*m*-Cresol was reported to be used at 2.4% in hair dyes and colors according to a survey performed by industry.

Under European regulations for cosmetic ingredients, 6-Amino-*m*-Cresol is categorized in Annex II, the list of substances prohibited in cosmetic products in Europe. The European Union's SCCS determined that 6-Amino-*m*-Cresol could not be considered safe for hair dying purposes due to the genotoxic potential of this ingredient and its metabolite, *N*-acetyl-2-amino-5-methylphenol. This determination was coupled with data that indicated that a dimer of the ingredient was formed under oxidative conditions and was found to absorb in human skin (in vitro).

In dermal penetration studies, the dermal delivery of an oxidative hair dye formulation containing a final concentration of 0.75% 6-Amino-*m*-Cresol was determined to be 0.34% in an in vitro study. In an in vivo study in rats, approximately 14.25% of the applied dose of 6-Amino-*m*-Cresol dissolved in DMSO (150 mg/ml) and 0.58% of the applied dose of a hair dye formulation containing 15 mg/g 6-Amino-*m*-Cresol were excreted, mainly in the urine. The remaining applied doses were recovered in the dressing washing, and application sites.

Toxicokinetics studies performed in vitro found 6-Amino-*m*-Cresol had the potential to be bioavailable after oral administration, and that metabolites may include *O*-glucurono-2-amino-5-methylphenol, 2-amino-5-methylphenol-*O*-sulfate, and *N*-acetyl-*O*-glucurono-2-amino-5-methylphenol. In rat ADME studies, 6-Amino-*m*-Cresol administered orally was well absorbed, readily distributed, extensively metabolized, and excreted mainly via urine. After dermal application, 5.1% of the radiolabeled dose was found in excretion, cage-wash, carcass, and unexposed skin. This amount increased to 6.8% when adding the residue in the exposed skin. Excretion was mainly in the urine, but elimination was slower compared to oral administration.

In an oral acute toxicity study in rats, the LD_{50} for 6-Amino-*m*-Cresol were calculated to be 1375 mg/kg bw in males and 1225 mg/kg bw in females. In mice, the LD_{50} were calculated to be 1020 mg/kg bw in male CF1 mice, 1225 mg/kg bw in female CF1 mice, and 750 mg/kg bw in CBL female mice.

An NOAEL could not be calculated in a 90-d oral gavage study in male and female rats that received a 10% suspension of 6-Amino-*m*-Cresol (98% pure) in 5% gum arabic at a dose of 800 mg/kg bw/d; after 6 wk, the dose was later reduced to 500 mg/kg bw/d due to clinical signs of toxicity and 2 animal deaths. Feed consumption, body weight, and body weight gains were significantly reduced in both sexes. Relative and absolute liver, kidney, and spleen weights were increased; however, no macroscopic or histopathological effects were observed.

6-Amino-*m*-Cresol (0-5000µg/plate in DMSO; 98.8% pure) was mutagenic in *S. typhimurium* strain TA100, with and without metabolic activation, but no mutagenicity was observed in strains TA98, TA102, TA1535, or TA1537. Clastogenic effects were observed in a concentration-dependent and biologically relevant manner in a mouse lymphoma L5178Y $tk^{+/-}$ cell gene mutation test of 6-Amino-*m*-Cresol (97.8% pure) at up to 100 µg/ml with metabolic activation. A biologically relevant increase in mutant frequency was not observed at up to 160 µg/ml without metabolic activation. 6-Amino-*m*-Cresol (98.8% pure) induced an increase in human lymphocytes with micronuclei when tested at up to 26.8 µg/ml without metabolic activation, but no genotoxicity was observed when tested at up to 67.7 µg/ml with metabolic activation. In an alkaline Comet assay, a concentration-dependent and biologically relevant increase in the amount of DNA in the tail was observed when 6-Amino-*m*-Cresol (98.8% pure) was tested at up to 1232 µg/ml, with and without metabolic activation. In a rat micronucleus test, 6-Amino-*m*-Cresol (single intraperitoneal injection of up to 400 m/kg bw; purity not reported) induced a biologically relevant and dose-dependent increase in the number of bone marrow cells with micronuclei in both sexes.

6-Amino-*m*-Cresol (purity not reported) was not irritating in guinea pigs when tested at 1% in water and thickened with methylcellulose. In a guinea pig sensitization test, no sensitization was observed to 3% 6-Amino-*m*-Cresol (purity not reported) in water and thickened with tylose. In an LLNA, 6-Amino-*m*-Cresol (98.9% pure) was a strong skin sensitizer when tested at up to 10% in DMSO (EC_3 of 3.44%) and at up to 5% in acetone:water (1:1) mixed with olive oil (3:1) (EC_3 of 1.55%). No ocular irritation was observed in guinea pigs to 1% 6-Amino-*m*-Cresol (aq.; purity not reported).

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.

Carcinogenicity studies on 6-Amino-*m*-Cresol were not included in the original report, were not found in the updated literature search, and unpublished data were not submitted.

DISCUSSION

In accordance with its Procedures, the Panel evaluates the conclusions of previously-issued reports approximately every 15 years. In 2004, the Panel published a final report on 6-Amino-*m*-Cresol and concluded that this ingredient was safe for use in oxidative and non-oxidative (semi-permanent) hair dyes. The report has been reopened due to 6-Amino-*m*-Cresol being banned for use in cosmetics by the European Commission. In this amended report, the Panel concluded that the available data are insufficient for determining the safety of this ingredient under the intended conditions of use as a hair colorant. The Panel noted a lack of relevant safety data and determined that the data needs from the Insufficient Data Announcement issued following the December 2022 Panel meeting remain unmet. In order to come to a conclusion of safety for this hair dye ingredient, the following additional data are needed:

- Method of manufacture
- In vivo genotoxicity studies

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.

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

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the available data are insufficient to make a determination of safety for 6-Amino-*m*-Cresol under the intended conditions of use as a hair dye ingredient.

TABLES

Table 1. Chemical properties of 6-Amino-*m*-Cresol

Property	Value	Reference
Physical Form	Beige to reddish-brown crystals	[2]
Molecular Weight (g/mol)	123.16	[2]
Density (g/ml @ 20 ºC)	0.77	[4]
Vapor pressure (mmHg @ 25 ºC)	0.00308 (estimated)	[4]
Sublimation Point (ºC)	156-159 (skips melting at 1 atm)	[4]
Water Solubility (g/l @ 20 ºC & pH 7.65)	5.9	[4]
Acetonitrile Solubility (g/l)	34	[4]
DMSO Solubility (g/l)	> 100	[4]
Acetone/Water Solubility (1:1; g/l)	33	[4]
log P_{ow}	1.14 (estimated)	[4]
UV Absorption (wavelength (λ)) (nm) in ethanol	210, 235, and 291	[2]

Table 2. Genotoxicity studies of 6-Amino-*m*-Cresol

Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
IN VITRO					
0, 100, 316, 1000, 2500, or 5000 µg/plate; 98.8% pure	DMSO	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	Ames test in accordance with OECD TG 471; with and without metabolic activation	Mutagenic in TA100, with and without metabolic activation; evidence of toxicity observed at and above 2500 µg/plate in all strains except for TA102, which started at 1000 µg/plate	[4]
0.1 - 160 µg/ml without metabolic activation; 0.5-100 µg/ml with metabolic activation; 97.8% pure	culture medium	mouse lymphoma L5178Y $tk^{+/-}$ cells	Mammalian cell gene mutation test in accordance with OECD TG 476; with and without metabolic activation	Clastogenic; concentration-dependent and biologically relevant increase in mutant frequency observed with metabolic activation, an increased occurrence of small colonies was found indicating a mutagenic or clastogenic effect; without metabolic activation, a biologically relevant increase in mutant frequency was not observed	[4]
8.6 - 26.8 µg/ml without metabolic activation; 25.0-67.7 µg/ml with metabolic activation; 98.8% pure	DMSO	human lymphocytes	Micronucleus test in accordance with OECD TG 487; with and without metabolic activation	Genotoxic; test material induced an increase in lymphocytes with micronuclei without metabolic activation; no biologically relevant increases in micronuclei observed with metabolic activation	[4]
25 – 1232 µg/ml without metabolic activation; 308-1232 µg/ml with metabolic activation; 98.8% pure	DMSO	V79 cells	Alkaline Comet assay; with and without metabolic activation	Genotoxic; concentration-dependent and biologically relevant increase in the amount of DNA in the tail observed with and without metabolic activation	[4]
IN VIVO					
0, 100, 200, or 400 mg/kg bw; purity not reported	2.5% hydroxypropylcellulose	Groups of 5 male and 5 female Crl: CD (SD)BR rats	Mammalian erythrocytes micronucleus test in accordance with OECD TG 474; single intraperitoneal injection	Genotoxic; test material induced a biologically relevant and dose-dependent increase in the number of bone marrow cells with micronuclei in both sexes	[4]

Table 3. Dermal irritation and sensitization studies of 6-Amino-*m*-Cresol

Concentration/Dose	Vehicle	Test Population	Procedure	Results	Reference
IRRITATION					
ANIMAL					
1% test material (purity not reported)	water, thickened with methylcellulose	10 female albino SPF guinea pigs	Dermal irritation study; test material applied on abraded skin on area of 3 cm x 4 cm; 3 times daily for 20 min for 2 d (consecutive)	Negligible erythema observed on day 1 that was not recognizable on day 2; no edema or crusts observed	[4]
SENSITIZATION					
ANIMAL					
3%; purity not reported	water, thickened with 0.5% tylose	15 female albino Pirbright SPF guinea pigs with additional 10 as controls	Magnusson and Kligman guinea pig sensitization study; test material applied to abraded flanks without occlusion; 5 d/wk for 3 wk	No erythema or edema up to 72 h after challenge	[4]
0.5, 1.5, 5, or 10%; test material 98.9% pure	DMSO or acetone: water (1:1) with olive oil (3:1)	Groups of 5 CBA/J female mice	LLNA in accordance with OECD TG 429; tested with 2 different vehicles: 6-Amino-*m*-Cresol tested with DMSO at up to 10%; with the acetone: water:olive oil mix at up to 5%; positive control was 1% *p*-phenylenediamine in DMSO	Strong skin sensitizer; EC_3 value in DMSO was 3.44%; EC_3 in acetone:water:olive oil mix was 1.55%	[4]

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