Amended Safety Assessment of 4-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., by **June 15, 2024**) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party, and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.*

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ABBREVIATIONS

ADME	absorption, distribution, metabolism, and excretion
A_{max}	absorbance maximum
AUC_{last}	area under the curve to last measurable plasma concentration
AUC_{∞}	area under to curve to infinity
C_{max}	maximum observed concentration
CHO	Chinese hamster ovary
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
DMSO	dimethyl sulfoxide
EC_3	estimated concentrations for a SI of 3
ECHA	European Chemicals Agency
EU	European Union
FD&C Act	Food, Drug, and Cosmetic Act
FDA	Food and Drug Administration
HPLC	high-performance liquid chromatography
HPLC-UV/RAD/Q-ToF	HPLC coupled to ultraviolet and radiochemical detection/quadrupole time-of flight
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LLNA	local lymph node assay
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MOS	margin of safety
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
SCCP	Scientific Committee on Consumer Products
SED	systemic exposure dose
SI	stimulation index
$t_{1/2}$	half-life
T_{max}	time to C_{max}
TG	test guideline
US	United States
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 4-Amino-*m*-Cresol, which is reported to function as an oxidative hair dye in cosmetic products. The Panel reviewed the available data to determine the safety of this ingredient. The Panel concluded that 4-Amino-*m*-Cresol is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

4-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 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 4-Amino-*m*-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 be presented as individual stand-alone reports.

Excerpts from the summaries of the previous report on 4-Amino-*m*-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.)

Most of the new data included in this safety assessment were found in the opinion of the Scientific Committee on Consumer Products (SCCP)[4] and in the dossier on 4-Amino-*m*-Cresol from the European Chemicals Agency (ECHA).[5] Please note that these sources provide summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when the SCCP and ECHA are cited.

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 January 2024. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the 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.

CHEMISTRY

Definition and Structure

4-Amino-*m*-Cresol is used as an oxidative hair dye.[4] According to the *Dictionary*, 4-Amino-*m*-Cresol (CAS No. 2835-99-6) is the substituted aromatic compound that conforms to the structure in Figure 1.[1] However, the use of regiochemical terms such as "*meta*-" (i.e., the "-*m*-" in 4-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 4-amino-3-methylphenol, is more common in the literature. Additionally, this ingredient may be supplied as a hemisulfate salt (i.e., 2 equivalents of 4-Amino-*m*-Cresol per 1 equivalent of sulfuric acid; no CAS No.).[4]

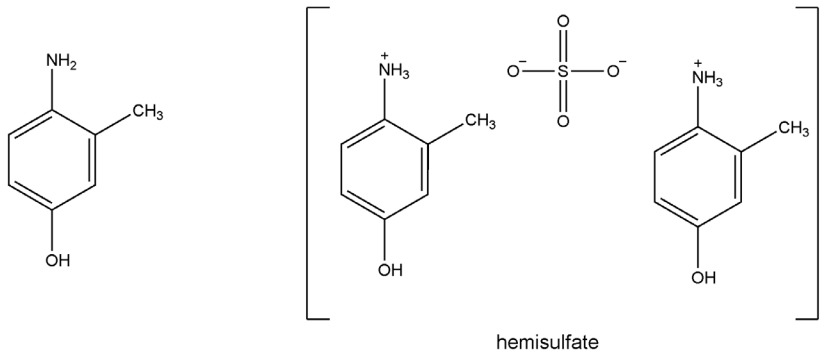

Figure 1. 4-Amino-*m*-Cresol

Chemical Properties

Chemical properties for 4-Amino-*m*-Cresol are summarized in Table 1. 4-Amino-*m*-Cresol (molecular weight 123 g/mol) has a log P_{ow} of 0.51.[4] 4-Amino-*m*-Cresol has a symmetrical absorption peak below 300 nm, with maxima at 206,

234, and 300 in ethanol.[2] The hemisulfate (formula weight of 344.48 g/mol) comprises 2 ammonium cations (i.e. 2 equivalents of "4-ammonium-*m*-cresol") and 1 divalent anion (sulfate), resulting in an estimated log P_{ow} of 0.89.[6]

Method of Manufacture

Method of manufacturing data for 4-Amino-*m*-Cresol were not included in the original report and were not found in the updated literature search, and unpublished data were not submitted. However, non-amino-substituted cresols are traditionally obtained via distillation of coal tar.[7]

Composition and Impurities

The impurity limits for 4-Amino-m-Cresol specify > 99.5% solid content, < 1.0% sulfated ash, and < 50 ppm iron, with assay of > 98.0%.[2] The typical analysis was > 99.9% solid content, < 0.5% sulfated ash, and < 10 ppm iron, with assay of 98.5% to 99.5%. No m-cresol was detected by high-performance liquid chromatography (HPLC).

The purity of 4-Amino-*m*-Cresol ranged from 94.5 - 99.8% (w/w) through nuclear magnetic resonance (NMR) spectroscopy of 9 different batches and 97.6 - 105.6% % (peak area) through HPLC.[4] Potential impurities of 4-Amino-*m*-Cresol include 3-methyl phenol, sulfanilic acid, and 4-nitro-3-methylphenol, which are raw materials for synthesis of the ingredient, were not detected via analysis.

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 their use in airbrush delivery systems. Data included herein were obtained from 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 were provided by cosmetic product categories, based at that time 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 data, 4-Amino-*m*-Cresol has 28 reported uses in hair dyes and colors.[8] The results of the concentration of use survey conducted by the Council in 2021 (provided in 2022) reported that the maximum concentration of use range of 4-Amino-*m*-Cresol is 0.08 - 0.14% in hair dyes and colors.[9] When the original safety assessment was published in 2004, 4-Amino-*m*-Cresol was reported to have no uses, according to 1998 VCRP data.[2] However, according to industry data from 1999, 4-Amino-*m*-Cresol was reported to be used at up to 0.3% in hair dyes and colors.

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 (FD&C 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.[10,11] 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 (EU), 4-Amino-*m*-Cresol is categorized in Annex III, the list of substances which cosmetic products must not contain except subject to the restrictions laid down.[12] For this ingredient, the regulation states that the maximum concentration applied to hair or eyelashes must not exceed 1.5% after mixing under oxidative conditions, and dyeing eyelashes is for professional use only. The Scientific Committee on Consumer Products (SCCP) concluded that

4_Amino-*m*-Cresol, at a maximum concentration of 1.5% in the finished cosmetic product (after mixing with hydrogen peroxide), does not pose a risk to the health of the consumer apart from its sensitizing potential.[4]

TOXICOKINETIC STUDIES

Dermal Absorption

In Vitro

The dermal absorption/percutaneous penetration potential of 4-Amino-*m*-Cresol (95.8% pure) through excised pig skin (840 µm thick) was determined from a commercial hair dye formulation.[4] The study was performed in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 428. The concentration of 4-Amino-*m*-Cresol in the aqueous cream formulation was 1.5%. Using Franz diffusion chambers, 1.5 mg of 4-Amino-*m*-Cresol was applied to the skin (400 mg formulation containing 1.5% 4-Amino-*m*-Cresol applied to 4 cm^2 skin) for 30 min. Application of the test material was terminated by gently rinsing with 4 ml water, then once with a diluted shampoo washing solution, and then twice again with water. The washing solutions were combined, and the amount of 4-Amino-*m*-Cresol was determined by HPLC. The receptor fluid (physiological phosphate buffer containing sodium chloride and antibiotics) was analyzed at 16 and 24 h post-application. At study end, the skin was extracted and 4-Amino-*m*-Cresol content was quantified.

The penetration rate was 0.7 to 1.1% of the applied dose. The total recovery was 96.1 $\pm$ 3.0% of the applied dose; preliminary stability tests showed that only about 5% of the initial quantity of the applied dose could be recovered after 16 h in the receptor fluid. This indicated a moderate to rapid decrease of 4-Amino-*m*-Cresol when added to the receptor fluid or brought into skin contact. The majority of 4-Amino-*m*-Cresol remained on the skin surface at 95.6 $\pm$ 3.1% of the applied dose. The quantification of the skin penetration by analysis of skin-extracts and the receptor fluid was found not meaningful. 4-Amino-*m*-Cresol was not detected in the receptor fluid. Thus, the limit of detection (equal to 132 ng/cm^2) was used to estimate the content for this compartment, i.e., 0.3 µg/cm^2 (equal to <0.02% of the applied dose). However, due to the instability of the test item in the receptor fluid, this approach was thought not to reflect real absorption conditions. Additionally, the content quantified in the skin as 8.0 ± 2.2 µg/cm^2 (equal to 0.5 ± 0.2 % of the applied dose) might not reflect the real conditions, due to the limited stability of the test item upon skin contact. The amount of 4-Amino-*m*-Cresol bioavailable from a commercial hair dye formulation was determined to range from 8.3 to 66.5 µg/cm^2, which is equivalent to 0.6 to 4.4% of the applied dose. The SCCP determined the study was inadequate based on the large variation in the bioavailability.[4]

In another dermal absorption study, [^{14}C]4-Amino-*m*-Cresol was studied at concentrations of 0.1, 0.5, 1.5, or 2% in formulation with hydrogen peroxide and a reaction partner using excised pig skin.[5] The study was performed in accordance with OECD TG 428. Approximately 400 mg of the test material was applied for 30 min to an exposure area of 4 cm^2. A total of 6 skin samples were used per concentration group for all but the 1.5% group (this group contained 12 samples). At the end of the exposure period, the samples were washed off with water and shampoo and the content of the test material in the rinsing solution was measured. The receptor fluid was sampled at 16, 24, 40, 48, 64, and 72 h intervals to determine the test material concentration. The skin samples were analyzed for radioactivity.

The majority of the test material was recovered in the rinsing solution (95.36, 91.86, 90.98, and 88.6% for the 0.1, 0.5, 1.5, and 2% concentration groups, respectively). Small amounts of radioactivity were detected in the upper skin, lower skin, and receptor fluid for each concentration tested. The total recovery ranged from 92.6% to 103.5%. For all concentrations tested, the material in the receptor fluid was predominantly detected within the first 16 h. The biologically available amounts of radiolabeled 4-Amino-*m*-Cresol were 0.061 $\pm$ 0.011, 0.313 $\pm$ 0.112, 0.858 $\pm$ 0.482, and 1.110 $\pm$ 0.611 µg/cm^2 (equivalent to 0.064, 0.068, 0.06, and 0.06%, respectively) for the 0.1, 0.5, 1.5, and 2% concentration groups, respectively.[5]

In a dermal absorption and metabolism study, [^{14}C]4-Amino-*m*-Cresol in a cream formulation mixed with a reaction partner was applied (150 mg) to human skin samples (thickness 0.387 to 0.695 µm) in 2-compartment static diffusion cells.[5] After an application period of 1 h, receptor fluid (Dulbecco's Minimum Eagle Medium and Ham F12 culture medium (3:1)) samples were collected at 3 h and 24 h to determine the amount of parent compound as well as the metabolic profile. The mean absorption of the test material was 0.26 $\pm$ 0.09% of dose applied (3.89 $\pm$ 1.37 µg/cm^2). Total recovery of the applied dose was 99.5 $\pm$ 1.7%. 4-Amino-*m*-Cresol was almost completely metabolized or converted, with 4-(acetylamino)-3-methyl-phenol seeming to account for a major portion of the profiled radioactivity.

Animal

Dermal absorption of [^{14}C]4-Amino-*m*-Cresol (hemisulfate) was studied in groups of 3 male and 3 female pigmented PVG rats.[4] The test substance was applied to an area of 9 cm^2 at concentrations of 15 % in dimethyl sulfoxide (DMSO) and of 1.5 % in a commercial formulation with hydrogen peroxide, for 24 h and 30 min contact, respectively. Mean dose applied was 1.611 mg/cm^2 for the material in DMSO and 1.516 mg/cm^2 for the commercial formulation. The treated skin was covered with an occlusive plaster during the exposure period. During the exposure time the treated skin area was securely sealed by an occlusive plaster. After the exposure period ended, the test substance was scraped off (formulation only) and the skin was rinsed with a shampoo formulation and warm water. After rinsing, the area was covered with an aluminum foil strip and securely sealed by an occlusive plaster to further prevent licking of the treated area during the 72 h in the metabolism cages. Details on systemic distribution and excretion are described in the following section under dermal animal studies.

Total recovery of the applied radioactivity was 86.7 and 89.8% for males and females, respectively, treated with the commercial formulation and 95.7 and 97.9 % for males and females, respectively, treated with the DMSO solution. The amount of radioactivity remaining on the skin for the cosmetic formulation represented 2.8 and 1.73% of the applied dose for males and females, respectively. For the DMSO solution, the radioactivity remaining on the skin was 7.86 and 6.1% of the applied dose for males and females, respectively. The majority of the applied doses were recovered in the dressings and the washing solutions, representing 83.3 - 87.7% and 74.4 - 81.6% of the applied amount for the formulation and the DMSO solution, respectively. A cutaneous absorption of 2.73% of applied dose (equivalent to 41.4 ug/cm²) was determined for a commercial formulation applied under typical use conditions in the presence of peroxide. The respective amount for the DMSO solution is 14.38 % of the applied dose (equivalent to 231.7 µg/cm²). No significant differences were noted between males and females with regard to skin absorption of 4-Amino-*m*-Cresol when applied in either a hair dye formulation or a DMSO solution.[4]

Absorption, Distribution, Metabolism, and Excretion (ADME) Studies

In Vitro

The metabolic profile of 4-Amino-*m*-Cresol was investigated in vitro using primary hepatocytes from male human donors, male Sprague Dawley rats, and male ICR/CD-1 mice.[4] The metabolic capacity of the hepatocytes was characterized by marker substrates for phase I (general cytochrome P450 activity for humans and rodents and specific activity of 1A1/2 and 2E1) and phase II enzymes (*N*-acetyl-transferase 1/2). Approximately 1 x 10^6 cells/ml were incubated with 10 µM (mouse hepatocytes) or 6.6 µM (rat, human hepatocytes) of 4-Amino-*m*-Cresol for 4 h. Samples of the supernatant were taken and analyzed at 0, 0.5, 1.5, and 4 h. The metabolic stability was assessed by detection of loss of parent compound by means of liquid chromatography with tandem mass spectrometry (LC-MS/MS). The metabolic profile was also studied using LC-MS and metabolites identified/characterized as far as possible. Cell viability (90%, 95% and 80% in human, rat, and mouse, respectively) was not affected by 4-Amino-*m*-Cresol during the incubation period. A slight decrease in viability of about 10% was noted for the end of the entire incubation period. The marker enzymes demonstrated the metabolic capacity and the validity of the test system. Differences in the metabolic capacity between rat, mouse and human hepatocytes were observed for the different phase I marker reactions. 4-Amino-*m*-Cresol revealed a rapid rate of metabolism in rat and human hepatocytes. A decrease of 95.2 and 89.8% of the parent compound was detected within 1.5 h incubation for human and rat hepatocytes, respectively. The mouse incubation could not be analyzed due to analytical problems. The analysis of the formed metabolites revealed an intensive phase II metabolism resulting in sulfation of the phenol group for all three species. In contrast, no indication of *N*-acetylation was noted for rat or human hepatocytes. The findings from studies on rat and human hepatocytes, under the same experimental conditions, show no significant difference in metabolic rate/capacity or metabolic profiles between these cells. The authors stated the outcomes of this comparative in vitro metabolism investigation in hepatocytes support the applicability of extrapolating data from rat data to humans in terms of liver metabolism.

A similar metabolic profile study was performed using human cryopreserved hepatocytes that were either suspended or plated prior to exposure to 4-Amino-m-Cresol.[5] The concentrations tested in the suspended hepatocytes was 1 or 10 µg/ml 4-Amino-m-Cresol, and in the plated hepatocytes, the concentrations tested were 0.862, 8.62, or 24.6 µg/ml 4-Amino-m-Cresol. Testing was performed in triplicate. The suspended cells were incubated for 3 h and the plated cells were incubated for 24 h. The formation of the metabolites was analyzed by high-performance liquid chromatography-coupled to ultraviolet and radiochemical detection/quadrupole-time-of flight (HPLC-UV/RAD/Q-ToF)-mass spectrometry. In the suspended hepatocytes, a glucuronide metabolite of 4-Amino-*m*-Cresol was formed, but it could not be determined if it was conjugated to *N* or *O*. A 10-fold increase in the test material dose did not result in a 10-fold increase in the glucuronide metabolite production, indicating the enzyme might have become saturated. In the plated hepatocytes, *N*-acetyl-4-amino-*m*-cresol and glucuronide metabolites of 4-Amino-*m*-Cresol were observed. Lower levels of metabolites were produced at the high concentration compared to the metabolites produced at the lower 2 concentrations, which may have been due to adverse effects on the hepatocytes at the highest concentration tested. The positive control yielded expected results.

In another similar study using immortalized human keratinocyte HaCaT cells, 4-Amino-*m*-Cresol was tested at 0.862, 8.62, and 24.6 µg/ml.[5] The cells were incubated after dosing for 3 or 24 h. One metabolite, *N*-acetyl-4-amino-*m*-cresol, was observed in the incubations at both 3 and 24 h intervals in all test concentrations.

In an in vitro study, the bioavailability of 4-Amino-*m*-Cresol across the intestinal barrier was investigated in human intestinal epithelial (TC-7) cells.[4] The permeability from the apical to the basolateral side was studied in 96- transwell plates with shaking for a 60 min contact time. Analysis of the donor (apical) and receiver (basolateral) samples was done by means of HPLC-MS/MS and the apparent permeability coefficient (P_{app}) was calculated for 2 independent experiments. Approximately 4 µM [^{14}C]mannitol was used to demonstrate the integrity of the cell monolayer. Only monolayers revealing a permeability of < 2.5 x 10^{-6} cm/sec were used. The validity of the test system was demonstrated by analyzing propranolol, vinblastine, and ranitidine. According to the laboratory's classification system, a low permeability is considered for test items revealing a P_{app} < 2 x 10^{-6} cm/sec. A P_{app} of 2 - 20 x 10^{-6} cm/sec and a P_{app} ≥ 20 x 10^{-6} cm/sec classify a substance to have a moderate and a high permeability, respectively. Ranitidine was used as a low permeability reference compound (it has a 50 % absorption in humans with a P_{app} of 0.4 x 10^{-6} cm/sec in the test). The total recovery for the reference substances and 4-Amino-*m*-Cresol ranged from 83 to 100%. A P_{app} of 59 x 10^{-6} cm/sec was determined for 4-Amino-*m*-Cresol, and thus was classified to be of high permeability, indicating nearly 100% absorption from the gastrointestinal tract.

Animal

Dermal

The dermal absorption study of [^{14}C]4-Amino-*m*-Cresol (hemisulfate) described in the above section also studied the excretion of the test material in the groups of 3 male and 3 female pigmented PVG rats.[4] The test substance was applied to an area of 9 cm^2 at concentrations of 15 % in DMSO and of 1.5 % in a commercial formulation with hydrogen peroxide, for 24 h and 30 min contact, respectively. Mean dose applied was 1.611 mg/cm^2 for the material in DMSO and 1.516 mg/cm^2 for the commercial formulation. Urine and feces were collected daily (0 - 24, 24 - 48, and 48 - 72 h after administration) from the cages. Exhaled carbon dioxide was removed every 24 h for the 72-h post-dosing period. Animals were killed 72 h after application, and the application sites, blood, and organs were analyzed for radioactivity. The radioactivity in the remaining carcass was also determined.

Total recovery of the applied radioactivity was 86.7 and 89.8% and 95.7 and 97.9% for males and females treated with the commercial formulation and the DMSO solution, respectively. Absorbed radioactivity was mainly excreted via urine both for the commercial formulation (0.35% males, 0.15% females) and for the DMSO solution (8.09% males, 5.00% females), with 79.4 - 88.9% of the total amount being excreted within the first 24 h. Excretion via feces was 0.01 - 0.02% for the formulation and 0.27 - 0.58% for the DMSO solution. Elimination via expiration was less than 0.2% for both the commercial formulation and the DMSO solution. Low levels of radioactivity were detected in all tissues examined for the DMSO solution, except for the thyroid, adrenal and gonads, in which detectable amounts were only found in one sex and/or for some but not all of the animals within one group. The highest levels were noted for the remaining carcass (0.059 - 0.063% of the applied dose) and the gastrointestinal tract content (0.006 - 0.007% of the applied dose). Similar findings were noted for the formulation, but the tissue levels were in general lower than the ones noted for the DMSO solution. Again, the highest levels were noted for the remaining carcass and the gastrointestinal-tract content as observed with the DMSO solution. No significant differences were noted between males and females with regard to tissue-distribution and elimination of 4-Amino-*m*-Cresol when applied with in either a hair dye formulation or a DMSO solution.[4]

The ADME and toxicokinetics of 4-Amino-*m*-Cresol was studied in female Wistar rats.[5] Groups of 4 - 6 rats received 12 or 60 mg/kg bw radiolabeled 4-Amino-*m*-Cresol (0.2 ml) either in cream formulation or DMSO applied uniformly onto shaved skin. The study was performed in accordance with OECD TG 417 and TG 427. O-rings were utilized in the application of the test material and the total dosing surface area was 20 cm^2 per animal. Animals wore a collar to prevent disruption of the treatment area. Exposure duration was 0.5 h in the low dose group and 24 h in the high dose group. The test substance was washed from the skin at the end of the exposure period and the application sites were tape-stripped. Animals were observed for mortality, clinical signs, and body weight before they were killed. Urine and feces were collected from the ADME group pre-dosing and during 4 intervals over 72 h. Cage rinses, wash, O-rings, and tape strips were collected at termination. Blood and tissue samples for the ADME group were collected after the animals were killed. Blood samples from the toxicokinetics group were collected at 0.25, 0.5, 0.75, 1, 2, 4, 8, 24, 48, and 72 h after dosing. Total percent radioactivity recovered was determined in the ADME group. For the toxicokinetic group, maximum observed concentration (C_{max}), time to C_{max} (T_{max}), half-life ($t_{1/2}$), area under the curve to last measurable plasma concentration (AUC_{last}), area under the curve to infinity (AUC_{∞}), and elimination rate were determined from plasma kinetics.

Body weights of all animals were within 20% of the sex mean. No unscheduled deaths were observed. All rats displayed red discharge from the nose and eye after dosing, which was related to wearing the collar. On average, 86 and 54% of the applied radioactivity was recovered from back-wash in the ADME low and high dose group, respectively. The average dermal absorption was 1.05% (or 0.001 mg/cm^2) in the low-dose group (cream formulation after 30 min) and 36.3%, (or 0.28 mg/cm^2) in high-dose group (DMSO after 24 h). In the high-dose group, the average total remaining radioactivity in blood, carcass, and tissues (excluding treated skin) was 0.98% of the administered dose, indicating no major accumulation of test substance after 72 h. The radioactivity detected in treated skin was 2.11% of the administered dose. In the low-dose group, radioactivity was below the detection limit in most tissues, and 0.4% of the administered radioactivity was excreted in urine, indicating poor dermal absorption. In the high-dose group, urinary excretion accounted for 32% of the administered dose, indicating urine was an important route of elimination for the test substance. The highest rate of excretion was observed during the first 8 h and decreasing excretion rate was noted thereafter. The fecal excretion of radioactivity was 0.2% after low dermal dosing and 2.4% after high dermal dosing. No toxicokinetic evaluation was performed for the low dose group, since most plasma data were below the limit of quantification. The toxicokinetic parameters determined from the high-dose toxicokinetics group were as follows: T_{max} value of 2 h, C_{max} was 15.7 mg/kg, AUC_{last} was 81.3 h x mg/kg, AUC_{∞} was 87.4 h x mg/kg, and $t_{1/2}$ was 30.1 h. The elimination rate was determined to be 0.023/h.[5]

Oral

The ADME and toxicokinetics of 4-Amino-*m*-Cresol following oral dosing was studied in female Wistar rats.[5] Groups of 4 - 6 rats received a single oral dose of 60 mg/kg bw radiolabeled 4-Amino-*m*-Cresol via gavage. The study was performed in accordance with OECD TG 417. The vehicle for the test material was 0.5% w/w carboxymethylcellulose and 0.5% w/w ascorbic acid in 0.05 M phosphate buffer. Animals were observed for mortality, clinical signs, and body weight before they were killed. Urine and feces were collected from the ADME group pre-dosing and during 4 intervals over 72 h. Cage rinses were collected at termination. Blood and tissue samples for the ADME group were collected after the animals were killed. Blood samples from the toxicokinetics group were collected at 0.25, 0.5, 0.75, 1, 2, 4, 8, 24, 48, and 72 h after

dosing. Total percent radioactivity recovered was determined in the ADME group. For the toxicokinetic group, T_{max}, C_{max}, $t_{1/2}$, AUC_{last}, AUC_{∞}, and elimination rate were determined from plasma kinetics.

Body weights of all animals were within 20% of the sex mean. No unscheduled deaths were observed. All rats displayed piloerection and brown discoloration of urine after dosing. Excretion in the urine accounted for 92% of the administered dose, with the highest rate of excretion observed during the first 8 h and decreasing excretion rate noted thereafter. Excretion in the feces was 3.9% of the administered dose and was delayed compared to urine, with the highest excretion between 8 and 24 h. Approximately 2.1% radioactivity was recovered in cage wash. The oral absorption, calculated as fractional absorption from the urine data, was 105%. The absolute oral bioavailability calculated from the plasma data was 86%. No major accumulation of radioactivity was observed at 72 h, with the average total radioactivity in blood, carcass, and tissues as 0.82% of administered dose. The highest residual concentration was observed in the liver. From the plasma data, with T_{max} value of 0.25 h showed that oral absorption was fast. C_{max} was 89.1 mg/kg, AUC_{last} was 163 h x mg/kg, AUC_{∞} was 167 h x mg/kg, and $t_{1/2}$ was 24 h. The elimination rate was determined to be 0.0289/h.[5]

Other Route

The same research study that studied the dermal and oral ADME and toxicokinetics of 4-Amino-*m*-Cresol in female Wistar rats described above also investigated an intravenous administration of 60 mg/kg bw of the test material.[5] Groups of 4 - 6 animals were injected once with 12 mg/ml test substance in 0.5% w/w carboxymethylcellulose and 0.5% w/w ascorbic acid in 0.05 M phosphate buffer. The remaining methodology is the same as described above for the oral study.

Body weights of all animals were within 20% of the sex mean. No unscheduled deaths were observed. All rats displayed piloerection and brown discoloration of urine after dosing. Excretion in the urine accounted for 88% of the administered dose, with the highest rate of excretion observed during the first 8 h and decreasing excretion rate noted thereafter. Excretion in the feces was 2% of the administered dose and was delayed compared to urine, with the highest excretion between 8 and 24 h. Approximately 2.6% radioactivity was recovered in cage wash. No major accumulation of radioactivity was observed at 72 h, with the average total radioactivity in blood, carcass, and tissues as 1% of administered dose. The highest residual concentration was observed in the liver. From the plasma data, the T_{max} value of 0.25 h showed that intravenous absorption was fast. C_{max} was 89.1 mg/kg, AUC_{last} was 3.48 h x mg/kg, AUC_{∞} was 197 h x mg/kg, and $t_{1/2}$ was 19 h. The elimination rate was determined to be 0.0364/h.[5]

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

In an acute toxicity study, groups of 6 female CF-1 mice and groups of 5 male and 5 female Wistar rats received single oral doses of 10% 4-Amino-*m*-Cresol in gum arabica via gavage.[4] The doses for the female mice were 750, 800, 850, 900, 950, or 1000 mg/kg bw. The male rats received 700, 800, 900, 1000, or 1100 mg/kg bw, while the female rats received 800, 900, 1000, 1100, or 1200 mg/kg bw. Mortality and clinical signs of toxicity were checked daily for 14 d. Body weights were recorded, and all animals underwent gross necropsy at the end of the observation period. Reduction of physical activity was noted in the animals. Deaths occurred within 2 to 48 h of dosing (number per group not reported). At necropsy, no macroscopic organ changes were observed. The LD_{50} for 4-Amino-*m*-Cresol in the female mice was 908 mg/kg bw. The LD_{50} in female rats was 1010 mg/kg bw, and in male rats, the LD_{50} was 870 mg/kg bw.

Short-Term Toxicity Studies

In a short-term oral toxicity study, groups of 6 male CD-1 mice received 1000, 1200, 1440, 1728, or 2074 mg/kg 4-Amino-m-Cresol (method of oral administration not specified) on 2 consecutive days.[2] The LD_{50} was determined as 1000 mg/kg. At least 1 mouse in all groups survived until day 14 of the observation period, but most mice died on day 1 or 2. Clinical observations included piloerection in all groups, hypokinesia in all but the low-dose group, ataxia in the 1440- and 2074-mg/kg dose groups, and prostration in the 1200 mg/kg dose group.

Subchronic Toxicity Studies

Oral

In a 13-wk oral study, male and female Wistar rats received 4-Amino-m-Cresol by gavage at doses of 0, 15, 60, or 120 mg/kg/d.[2] No clinical observations or pathological findings indicative of systemic toxicity were observed in the 15 mg/kg dose group. The 60 and 120 mg/kg dose groups had dark, discolored urine from weeks 8 to 13 that were attributed to the test material. The 120 mg/kg dose group had significantly increased creatinine values in female rats after 13 wk, although values were still within the normal range. Absolute spleen weights were increased in a statistically significant manner in female rats in the 120 mg/kg dose group (absolute spleen weights were also increased in males, but not in a statistically significant manner). No microscopic changes attributed to the test material were observed. The no-observed-adverse-effect-level (NOAEL) was 60 mg/kg/d.

Repeated-dose toxicity studies of 4-Amino-*m*-Cresol were not found in the updated literature search, and unpublished data were not submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

Groups of 24 female rats (strain BOR:WISW-SPF TNO) were dose orally with 0, 10, 40, or 80 mg/kg 4-Amino-m-Cresol from days 5 to 15 of gestation.[2] Dams were killed on day 20 of gestation. No clinical signs of toxicity nor mortalities were observed in the dams. No abnormalities were observed at gross necropsy. No abnormalities were observed in litter parameters. No malformations were observed in external or skeletal examination of fetuses. Hydrocephaly was observed in 1 fetus of the 40 mg/kg dose group and minor visceral anomalies (increased renal pelvic cavitation) was observed in 2 fetuses in the 80 mg/kg dose group. The malformation index for all groups was 0, except the 40 mg/kg dose group, which had a malformation index of 0.56%. The NOAEL was established at 80 mg/kg.

Development and reproductive toxicity studies for 4-Amino-*m*-Cresol were not found in the updated literature search, and unpublished data were not submitted.

GENOTOXICITY STUDIES

4-Amino-m-Cresol was not mutagenic with or without metabolic activation in an Ames test evaluating concentrations of up to 600 µg/plate, alone and with equal amounts of 6% hydrogen peroxide.[2] 4-Amino-m-Cresol in DMSO did not induce unscheduled DNA synthesis in in vitro male rat primary hepatocytes at up to 100 µg/ml. In in vivo micronucleus tests in mice, 4-Amino-m-Cresol in DMSO or carboxymethylcellulose did not induce micronuclei after a single oral dose of up to 1000 mg/kg bw. In Chinese hamsters, 4-Amino-m-Cresol (hemisulfate) in double distilled water did not cause sister chromatid exchanges at oral doses up to 2000 mg/kg or intraperitoneal doses up to 400 mg/kg. No unscheduled DNA synthesis was induced in rats that received up to 1000 mg/kg 4-Amino-m-Cresol.

Additional in vitro and in vivo genotoxicity studies on 4-Amino-*m*-Cresol summarized here are detailed in Table 2. In Ames tests, 4-Amino-*m*-Cresol was not mutagenic, with or without metabolic activation, at up to 5000 µg/plate.[4,5] 4-Amino-*m*-Cresol (free base and hemisulfate) was not genotoxic in a L5178Y mouse lymphoma cell assays at the *tk* locus at up to 6.25 µg/ml without metabolic activation or at up to 1000 µg/ml with metabolic activation. Clastogenic effects were observed in chromosome aberration tests with 4-Amino-*m*-Cresol (free base and hemisulfate) in human peripheral lymphocytes (tested at up to 100 µg/ml without metabolic activation and at up to 156.25 µg/ml with metabolic activation) and in Chinese hamster ovary (CHO) cells (tested at up to 500 µg/ml without metabolic activation and at up to 2000 µg/ml with metabolic activation).[5] Ambiguous results for genotoxicity were observed in a micronucleus test using human peripheral lymphocytes exposed to up to 150 µg/ml 4-Amino-*m*-Cresol with metabolic activation; without metabolic activation, the test material was not genotoxic. Ambiguous results for genotoxicity were also observed in an unscheduled DNA synthesis assay in rat primary hepatocytes exposed to up to 10.0 µg/ml 4-Amino-*m*-Cresol. In HeLa cells, genotoxicity was observed in an unscheduled DNA synthesis assay of 4-Amino-*m*-Cresol (hemisulfate) at up to 500 µg/ml.

An in vivo micronucleus test found that 4-Amino-*m*-Cresol did not induce micronuclei in mice that received a single intraperitoneal dose of up to 200 mg/kg bw.[4] Unscheduled DNA synthesis was not observed in the hepatocytes of male rats that received a single oral dose of up to 2000 mg/kg bw 4-Amino-*m*-Cresol;[4] however, genotoxicity was observed in the same type of assay performed with up to 600 mg/kg 4-Amino-*m*-Cresol (hemisulfate).[5]

CARCINOGENICITY STUDIES

Carcinogenicity data for 4-Amino-*m*-Cresol 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

Dermal irritation and sensitization data on 4-Amino-*m*-Cresol are summarized in Table 3. Undiluted 4-Amino-*m*-Cresol was classified as a non-irritant and non-corrosive in EpiSkin™ and EpiDerm™ assays using human epidermal keratinocytes.[5] A 3% aqueous solution of 4-Amino-*m*-Cresol in 0.5% tylose was not irritating in a study with guinea pigs.[4] In a guinea pig maximization test, 4-Amino-*m*-Cresol was non-sensitizing when induced and challenged at up to 3%; however, the SCCS noted the test design of this study was inadequate and had reporting deficiencies. 4-Amino-*m*-Cresol was a moderate sensitizer in a local lymph node assay (LLNA) when tested at up to 10% in DMSO or up to 5.0% in water/acetone (1:1) mixed with olive oil (4:1). The estimated concentrations for a stimulation index (SI) of 3 (EC_3) were calculated to be 1.45% for the DMSO group and 2.15% for the water/acetone/oil group.

OCULAR IRRITATION STUDIES

In vitro and animal ocular irritation data on 4-Amino-*m*-Cresol are summarized in Table 4. Undiluted and 1.5% 4-Amino-*m*-Cresol in 50% propylene glycol were classified as not irritating in isolated chicken eye tests.[5] In a human cornea model test, undiluted 4-Amino-*m*-Cresol led to a viability of 93.8%; however, the test system was determined not suitable for assessment of the test material because the material was proved to be a strong reducer of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent. In an ocular irritation study using guinea pigs, 1.5% 4-Amino-*m*-Cresol in 50% propylene glycol was determined to have minimal ocular irritant potential.[4]

MARGIN OF SAFETY

The SCCP calculated the margin of safety (MOS) for 1.5% 4-Amino-*m*-Cresol in a formulation with hydrogen peroxide (on head) to be 124.[4] This calculation is based on the NOAEL of 60 mg/kg bw/d from a 90-d oral rat study and a systemic exposure dose (SED) of 0.483 mg/kg bw (skin area surface of 700 cm^2 x absorption through skin of 41.4 $\mu g/cm^2$ x 0.001 (unit conversion)/typical human bw of 60 kg).

The 2022 survey by the Council indicates that the highest usage concentration range for 4-Amino-*m*-Cresol in hair dye products is between 0.08 and 0.14%.[9] In a dermal penetration study using pig skin, bioavailability of 4-Amino-*m*-Cresol at a concentration of 0.5% in formulation with hydrogen peroxide was 0.068% (with a range of 0.064 to 0.06%, corresponding to 0.1 to 2% of the dosage applied).[5] Thus, an MOS calculation was performed under the assumption of 0.068% dermal absorption, the usage of 100 ml of permanent hair dye per application, and a retention factor of 0.1.[13] This yielded an SED value of 0.000158 mg/kg bw/d, based on the assumption that the average adult body weight is 60 kg. By using an NOAEL of 60 mg/kg bw/d derived from a 13-wk oral study,[2] the MOS was calculated to be approximately 380,000.

$$\text{MOS} = \frac{\text{NOAEL}}{\text{SED}} = \frac{60 \text{ mg/kg bw/d}}{0.000158 \text{ mg/kg bw/d}} = \sim 380{,}000$$

The resulting MOS is greater than 100, which is generally considered to be protective. The standard of MOS value of 100 is derived from multiplying two factors: a 10-fold factor accounts for the extrapolating data from test animals to human being (interspecies extrapolation), and an additional 10-fold for accommodating differences among the human population (intra-species extrapolation).

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. 4-Amino-*m*-Cresol is reported to be used in 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

4-Amino-*m*-Cresol is reported to function in cosmetics as a hair colorant. 4-Amino-*m*-Cresol was previously reviewed by the Panel as part of a safety assessment of 6 amino-cresol hair dye ingredients that was published in 2004. At that time, the Panel concluded that according to the available data (in that report), 4-Amino-*m*-Cresol is 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 assessment being banned for use in cosmetics by the European Commission.

According to 2023 VCRP survey data, 4-Amino-*m*-Cresol has 28 reported uses in hair dyes and colors. The results of the concentration of use survey conducted by the Council in 2021 (provided in 2022) reported that the maximum concentration of use range for 4-Amino-*m*-Cresol is 0.08 - 0.14% in hair dyes and colors. When the original safety assessment was published in 2004, 4-Amino-*m*-Cresol was reported to have no uses, according to 1998 VCRP data. However, according to industry data from 1999, 4-Amino-*m*-Cresol was reported to be used at up to 0.3% in hair dyes and colors.

According to the EU, 4-Amino-*m*-Cresol is categorized in Annex III, the list of substances which cosmetic products must not contain except subject to the restrictions laid down. For this ingredient, the regulation states that the maximum concentration applied to hair or eyelashes must not exceed 1.5% after mixing under oxidative conditions, and dyeing eyelashes is for professional use only. The SCCP concluded that 4-Amino-*m*-Cresol, at a maximum concentration of 1.5% in the finished cosmetic product (after mixing with hydrogen peroxide), does not pose a risk to the health of the consumer apart from its sensitizing potential.

In one in vitro dermal penetration study in excised pig skin, the bioavailability of 4-Amino-*m*-Cresol in a commercial hair dye formulation ranged from 8.3 to 66.5 $\mu g/cm^2$ (0.6 to 4.4% of the applied dose); however, the SCCP determined the study was inadequate based on the large variation of the bioavailability. In another in vitro study pig skin study of 4-Amino-*m*-Cresol, the ingredient was tested in formulation with hydrogen peroxide and a reaction partner at concentrations of 0.1, 0.5, 1.5, and 2%. The biologically available amounts of 4-Amino-*m*-Cresol were 0.061 ± 0.011, 0.313 ± 0.112, 0.858 ± 0.482, and 1.110 ± 0.611 $\mu g/cm^2$ (equivalent to 0.064, 0.068, 0.06, and 0.06%, respectively) for the 0.1, 0.5, 1.5, and 2%

concentration groups, respectively. In a dermal absorption and metabolism study using human skin samples, 4-Amino-*m*-Cresol in a cream formulation mixed with a reaction partner was completely metabolized and the mean absorption was 3.89 ± 1.37 µg/cm^2. The cutaneous absorption of 4-Amino-*m*-Cresol (hemisulfate) in rats was determined to be 41.4 ug/cm² (2.73% of applied dose) for a commercial formulation applied under typical use conditions in the presence of peroxide and 221.7 µg/cm^2 (14.38% of applied dose) for a DMSO solution.

In vitro metabolism studies found that 4-Amino-*m*-Cresol was rapidly metabolized in rat and human hepatocytes and yielded *N*-acetyl-4-amino-*m*-cresol and glucuronide metabolites. An in vitro absorption study using human intestinal epithelial cell line TC-7 cells indicated a good absorption of 4-Amino-*m*-Cresol after oral administration. In a rat dermal study of [^{14}C]4-Amino-*m*-Cresol (hemisulfate) at concentrations of 15 % in dimethyl sulfoxide (DMSO) and of 1.5 % in a commercial formulation with hydrogen peroxide, small amount of absorbed radioactivity was mainly excreted in urine, with 79.4-88.9% of the total amount being excreted within the first 24 h. Excretion via feces and respiration was at much lower percentages and little radioactivity was measured in the tissues. With dermal dosing of 12 or 60 mg/kg bw 4-Amino-*m*-Cresol in female rats, the majority of the applied dose was rinsed off. In the low dose group, 0.4% of administered radioactivity excreted in urine, indicating poor dermal absorption. In the high dose group, urinary excretion accounted for 32% of the administered dose, indicating urine as an important route of elimination for test substance. The highest rate of excretion was observed during the first 8 h and decreasing excretion rate noted thereafter. After oral administration to female Wistar Crl:WI rats, 4-Amino-*m*-Cresol was extensively absorbed (105% of the administered dose based on urine data and 86% based on plasma data), extensively metabolized and excreted mainly via the urine (92%). No major accumulation of the test material seems to occur in the body 72 hours after administration (0.82%). Intravenous administration of 4-Amino-*m*-Cresol to female rats showed that the majority of the administered dose was excreted in the urine within the first 8 h of dosing.

The LD_{50} for 4-Amino-*m*-Cresol (10%) in the female mice was 908 mg/kg bw. The LD_{50} for the same test material in female rats was 1010 mg/kg bw, and in male rats, the LD_{50} was 870 mg/kg bw.

In Ames tests, 4-Amino-*m*-Cresol was not mutagenic, with or without metabolic activation, at up to 5000 µg/plate. 4-Amino-*m*-Cresol (free base and hemisulfate) was not genotoxic in a L5178Y mouse lymphoma cell assays at the *tk* locus at up to 6.25 µg/ml without metabolic activation or with up to 1000 µg/ml with metabolic activation. Clastogenic effects were observed in chromosome aberration tests with 4-Amino-*m*-Cresol (free base and hemisulfate) in human peripheral lymphocytes (tested at up to 100 µg/ml without metabolic activation and at up to 156.25 µg/ml with metabolic activation) and in CHO cells (tested at up to 500 µg/ml without metabolic activation and at up to 2000 µg/ml with metabolic activation). Ambiguous results for genotoxicity were observed in a micronucleus test using human peripheral lymphocytes exposed to up to 150 µg/ml 4-Amino-*m*-Cresol with metabolic activation; without metabolic activation, the test material was not genotoxic. Ambiguous results for genotoxicity were also observed in an unscheduled DNA synthesis assay in rat primary hepatocytes exposed to up to 10.0 µg/ml 4-Amino-*m*-Cresol. In HeLa cells, genotoxicity was observed in an unscheduled DNA synthesis assay of 4-Amino-*m*-Cresol (hemisulfate) at up to 500 µg/ml.

An in vivo micronucleus test found that 4-Amino-*m*-Cresol did not induce micronuclei in mice that received a single intraperitoneal dose of up to 200 mg/kg bw. Unscheduled DNA synthesis was not observed in the hepatocytes of male rats that received a single oral dose of up to 2000 mg/kg bw 4-Amino-*m*-Cresol; however, genotoxicity was observed in the same type of assay performed with up to 600 mg/kg 4-Amino-*m*-Cresol (hemisulfate).

Undiluted 4-Amino-*m*-Cresol was classified as a non-irritant and non-corrosive in EpiSkin™ and EpiDerm™ assays using human epidermal keratinocytes. A 3% aqueous solution of 4-Amino-*m*-Cresol in 0.5% tylose was not irritating in a study with guinea pigs. In a guinea pig maximization test, 4-Amino-*m*-Cresol was non-sensitizing when induced and challenged at up to 3%; however, the SCCS noted the test design of this study was inadequate and had reporting deficiencies. 4-Amino-*m*-Cresol was a moderate sensitizer in an LLNA when tested at up to 10% in DMSO or up to 5.0% in water/acetone (1:1) mixed with olive oil (4:1). The EC_3 were calculated to be 1.45% for the DMSO group and 2.15% for the water/acetone/oil group.

Undiluted and 1.5% 4-Amino-*m*-Cresol in 50% propylene glycol were classified as not irritating in isolated chicken eye tests. In a human cornea model test, undiluted 4-Amino-*m*-Cresol led to a viability of 93.8%; however, the test system was determined not suitable for assessment of the test material because the material was proved to be a strong reducer of MTT reagent. In an ocular irritation study using guinea pigs, 1.5% 4-Amino-*m*-Cresol in 50% propylene glycol was determined to have minimal ocular irritant potential.

An MOS calculation was performed for 0.14% 4-Amino-*m*-Cresol; the MOS was approximately 380,000. This calculation is based on the NOAEL of 60 mg/kg bw/d from a 13-wk oral rat study and an SED of 0.000158 mg/kg bw/d. The resulting MOS is greater than 100, which is generally considered to be protective.

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

DISCUSSION

In accordance with its Procedures, the Panel re-evaluates the conclusions of previously-issued reports approximately every 15 years. In 2004, the Panel published a final report on 4-Amino-*m*-Cresol and concluded that the available data supported the safety of this ingredient as used in oxidative and nonoxidative (semi-permanent) hair dyes. This report was reopened because several of the other amino-cresol hair dye ingredients that were included in the original 2004 report are banned for use in cosmetics by the European Commission.

The Panel noted that this ingredient functions as an oxidative hair dye in hair coloring products. To estimate risk, an MOS calculation was performed for 4-Amino-*m*-Cresol using a dermal absorption rate of 0.068% for 0.14% 4-Amino-*m*-Cresol and an NOAEL of 60 mg/kg bw/d. The resulting MOS is approximately 380,000, which is considered protective. In vitro and in vivo genotoxicity studies yielded mixed results, and no carcinogenicity data were available; however, any concern was mitigated by the weight-of-evidence of negative results for other toxicity endpoints, including developmental and reproductive toxicity low dermal absorption, and negative results from dermal irritation and sensitization studies. The Panel noted the lack of method of manufacturing information, but data on composition and impurities for these ingredients and the high degree of reported purity obviated this need. 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 4-Amino-*m*-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 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 4-Amino-*m*-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 for 4-Amino-*m*-Cresol

Property	Value	Reference
Physical Form	Reddish-brown crystals	2
	Grey powder	4
Molecular Weight (g/mol)	123	2
Density (g/ml @ 20 ºC	1.24	5
Boiling Point (ºC)	not detectable (decomposition)	4
	156 - 190 (decomposition)	5
Melting Point (ºC)	178	2
Vapor Pressure (mmHg @ 20 ºC)	2.48×10^{-5}	4
Water Solubility (g/l @ 20 ºC)	Slightly soluble in water	2
(g/l @ 25 ºC)	2	5
log P_{ow}	0.51	4
UV Absorption (λ) (nm)	maxima at 206, 234, and 300 in ethanol	2
	symmetrical absorption peak at 300	

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
IN VITRO						
4-Amino-*m*-Cresol; purity not reported	Up to 600 µg/plate without metabolic activation; up to 3000 µg/plate with metabolic activation	ammonia, isopropanol, distilled water	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	Bacterial reverse mutation test in accordance with OECD TG 471; with and without metabolic activation	Not mutagenic, with or without metabolic activation	[5]
4-Amino-*m*-Cresol; HPLC purity: 97.8 area % (254 nm) and 99.2 area % (300 nm)	1 - 5000 µg/plate	DMSO	*S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	Bacterial reverse mutation test in accordance with OECD TG 471; with and without metabolic activation	Not mutagenic; test material did not induce gene mutations with or without metabolic activation; positive control (not reported) yielded expected results	[4]
4-Amino-*m*-Cresol; HPLC purity: 94.7 area %	Test 1: up to 6.25 µg/ml without metabolic activation Test 2: up to 47.5 µg/ml with metabolic activation Test 3: up to 40 µg/ml with metabolic activation	cell culture medium (RPMI with 3% horse serum)	L5178Y mouse lymphoma cells	Mammalian cell gene mutation test at the *tk* locus in accordance with OECD TG 476; with and without metabolic activation	Not mutagenic. In Test 1, slightly increased mutant frequency observed at 3.125 and 6.25 µg/ml; however, a strong cytotoxic effect was observed at 6.25 µg/ml. In Test 2, a slight increase in mutant frequency was measured for 25 and 47.5 µg/ml. In Test 3, no induction of mutants was observed at up to 30 µg/ml and a marginal effect was measured at 40 µg/ml Negative and positive controls (not reported) were in accordance with the guideline.	[4]
4-Amino-*m*-Cresol (hemisulfate); purity not reported	Up to 5 µg/ml without metabolic activation; up to 1000 µg/ml with metabolic activation	DMSO	L5178Y mouse lymphoma cells	Mammalian cell gene mutation test at the *tk* locus in accordance with OECD TG 476; with and without metabolic activation	Not mutagenic. With metabolic activation, high mutation frequencies observed in one replicate at 125 µg/ml and in both replicates at 1000 µg/ml as compared the solvent control. However, mutation frequencies at other test concentrations (63, 250 and 500 µg/ml) overlapped with the solvent control and regression analysis showed there was no clear dose-related response.	[5]
4-Amino-*m*-Cresol; purity not reported	Up to 20 µg/ml without metabolic activation; up to 156.25 µg/ml with metabolic activation	DMSO	human peripheral lymphocytes	Chromosome aberration test in accordance with OECD TG 473; with and without metabolic activation; negative and positive controls were used	Clastogenic with and without metabolic activation; test material induced significant chromosomal aberrations	[5]
4-Amino-*m*-Cresol (hemisulfate); purity not reported	up to 100 µg/ml with and without metabolic activation	Dulbecco's modified eagle medium/Ham's F12 medium (1:1)	human peripheral lymphocytes	Chromosome aberration test in accordance with OECD TG 473; with and without metabolic activation; negative and positive controls were used	Genotoxic; structural chromosome aberrations were induced, with and without metabolic activation	[5]
4-Amino-*m*-Cresol (hemisulfate); purity not reported	Up to 500 µg/ml without metabolic activation; up to 2000 µg/ml with metabolic activation	anhydrous DMSO	CHO cells	Chromosomal aberration test in accordance with OECD TG 473; with and without metabolic activation; negative and positive controls were used	Clastogenic with and without metabolic activation; test material induced statistically significant and dose-related increases in cultured CHO cells	[5]

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
4-Amino-*m*-Cresol; purity not reported	Test 1: up to 30 µg/ml without metabolic activation; up to 150 µg/ml with metabolic activation Test 2: up to 35 µg/ml without metabolic activation; up to 125 µg/ml with metabolic activation	DMSO	human peripheral lymphocytes	Micronucleus test in accordance with OECD TG 487; with and without metabolic activation; negative and positive controls were used	With metabolic activation, ambiguous results were observed; statistically higher frequencies of micronuclei binucleate were observed in the 3 highest concentrations when compared to vehicle controls. Single cultures exposed to 100 and 150 µg/ml of test material exceeded historical vehicle control range; however, overall mean micronuclei binucleate frequency fell within historical control range. Without metabolic activation, test material was not genotoxic	[5]
4-Amino-*m*-Cresol; purity not reported	0.10, 0.33, 1.00, 3.33, or 10.00 µg/ml	DMSO	rat primary hepatocytes	Unscheduled DNA synthesis assay, with metabolic activation only; 2 tests run in parallel; cells treated for 18 h with test material with [^{3}H]thymidine; positive and negative controls used	Ambiguous results. In Test 1, increased nuclear and net grain counts were observed. In Test 2, no reproductible concentration-dependent increase in the number of nuclear grain counts and net grain counts up to the highest concentration were observed.	[5]
4-Amino-*m*-Cresol (hemisulfate); purity not reported	Up to 500 µg/ml with and without metabolic activation	DMSO	HeLa cells	Unscheduled DNA synthesis assay; with and without metabolic activation; cells treated with tests substance or positive controls plus [^{3}H]thymidine; after incubation for 2.5 h, DNA isolated and quantified	Genotoxic; induced unscheduled DNA synthesis, with and without metabolic activation	[5]
IN VIVO						
4-Amino-*m*-Cresol; HPLC purity: 94.7 area %	20, 100, or 200 mg/kg bw	0.9% sodium chloride	5 male and 5 female NMRI mice per dose group	Mammalian bone marrow micronucleus test in accordance with OECD TG 474; single intraperitoneal dose; groups of animals killed at 24 h (all doses) or 48 h (200 mg/kg only) post-treatment; appropriate negative and positive controls used. No additional detail was available on the timing of the bone marrow extraction.	Not genotoxic; test material did not induce chromosome aberrations or damage to the mitotic apparatus in bone marrow cells of mice. In all treated groups, the relative polychromatic erythrocyte frequency was not decreased; the highest dose tested induced signs of toxicity (palpebral closure, lethargy) within 1 h of administration. No toxic effects observed at later time points. Positive control yielded expected results.	[4]
4-Amino-*m*-Cresol (hemisulfate); purity not reported	70, 200, or 600 mg/kg	distilled water	groups of 6 male and 6 female Wistar rats	Unscheduled DNA synthesis assay in accordance with OECD TG 486; rats received single gavage treatment and were killed 14 h post-administration; liver cells isolated and DNA analyzed; appropriate negative and positive controls used	Genotoxic; mean silver grain count was increased significantly compared to negative control in all dose groups; the amount of induced cells in the 200 and 600 mg/kg groups was significantly higher than negative control	[5]
4-Amino-*m*-Cresol; > 99% purity	60, 600, or 2000 mg/kg bw	DMSO/PEG 400	3 male Wistar/WU rats per dose group	Rat liver in vivo/in vitro unscheduled DNA synthesis assay; single gavage dose; rat hepatocytes studied in vitro after animals dosed in vivo; at 16 h, the 600 mg/kg dose group was killed and at 4 h, the 60 and 1000 mg/kg dose groups were killed; appropriate negative and positive controls used	Not genotoxic. No toxicity observed in any treated animals. No significant induction of unscheduled DNA synthesis compared to negative control group, no significant differences in the viability of hepatocytes in any dose group. Controls yielded expected results. The SCCS noted the study did not meet the requirements of the actual guideline.	[4]

Table 3. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
IRRITATION						
IN VITRO						
4-Amino-*m*-Cresol; purity not reported	none	undiluted; 10 mg	human epidermal keratinocytes	Skin irritation potential study using the EpiSkin™ reconstructed human epidermis model in accordance with OECD TG 439; 15 min treatment followed by post-exposure incubation period of 42 h; concurrent positive and negative controls utilized	Classified as a non-irritant; test material induced a relative mean viability at 63.3% compared to negative control	[5]
4-Amino-*m*-Cresol; purity not reported	none	undiluted; 25 mg	human epidermal keratinocytes	Skin corrosivity potential study using the EpiDerm™ human skin model in accordance with OECD TG 431; treatment periods of 3 and 60 min; concurrent positive and negative controls utilized	Classified as non-corrosive; test material did not induce decrease of tissue viability; 90.1% viability after 3 min and 80.8% viability after 60 min	[5]
ANIMAL						
4-Amino-*m*-Cresol; purity not reported	0.5% tylose	3% aqueous solution	15 female Pirbright White guinea pigs	Skin irritation study; test material applied daily by brush for 5 d on a 3 cm x 4 cm test area; test sites not occluded but animals were restrained for 5 h post-application; examinations for erythema and edema performed at 5 h post-application	Non-irritating; no skin reactions observed at any observation time point; no clinical signs of toxicity observed	[4]
SENSITIZATION						
ANIMAL						
4-Amino-*m*-Cresol; purity not reported	distilled water	induction: 3% challenge: 1, 2, and 3%	test group: 20 Pirbright Hoe:DKPK guinea pigs control group: 10 guinea pigsgroup	Guinea pig maximization test; first intradermal induction with 0.05 ml of test material in distilled water with Freund's complete adjuvant/arachidis oil injected in parallel; after 6 to 8 h, animals pre-treated with 10% sodium lauryl sulfate in pet. and topical induction with 5 ml of test material in pet. on 2 cm x 4 cm test area on flank, test site occluded for 24 h; second intradermal induction with test material in Freund's complete adjuvant/arachis oil; challenge phase started on day 16 of study with patches of 0.5 ml of test material in Freund's complete adjuvant/arachis oil under occlusion for 24 h; test sites evaluated after 24 and 48 h; concurrent vehicle and positive (1-chloro-2,4-dinitrobenzne) control groups were used	Non-sensitizing; no dermal irritation observed during the induction phase and no dermal sensitization observed during the challenge phase; controls yielded expected results The SCCS noted the test design of this study was inadequate and had reporting deficiencies	[4]
4-Amino-*m*-Cresol; 95.8% pure	DMSO or aqua/acetone (1:1) mixed with olive oil (4:1)	DMSO: 0, 0.5, 1.5, 5.0, or 10% water/acetone/oil: 0, 0.5, 1.5, 3.0, or 5.0%	groups of 5 female CBA/J mice	LLNA; mice received test material (25 µl) on ear surface once daily for 4 d; p-Phenylenediamine (1%) in DMSO was positive control; 5 d after 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	Moderate sensitizer; no abnormal signs of toxicity or mortality during study. Mean SI were calculated to be 0.9, 3.1, 6.5, and 6.7 for the 0.5, 1.5, 5.0, and 10% dose groups in DMSO, respectively; estimated concentration for the EC_3 was calculated to be 1.45%. For the water/acetone/olive oil groups, the SI were calculated to be 1.5, 1.7, 4.7, and 6.9 for the 0.5, 1.5, 3, and 5% dose groups, respectively. EC_3 for this group was calculated to be 2.15%. Controls yielded expected results.	[4]

Table 4. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
			IN VITRO			
4-Amino-*m*-Cresol; purity not reported	50% propylene glycol	1.5% (w/w) dilution; 30 µl	3 eyes from Ross spring chickens	Isolated chicken eye test method in accordance with OECD TG 438; eyes exposed to single application of test material for 10 s and observed for up to 4 h; concurrent positive and negative controls utilized	Classified as not irritating	[5]
4-Amino-*m*-Cresol; purity not reported	none	undiluted; 30 mg	3 eyes from Ross spring chickens	Isolated chicken eye test method in accordance with OECD TG 438; eyes were exposed to a single application of test material for 10 s, rinsed with 20 ml saline, and observed for up to 4 h; concurrent positive and negative controls utilized	Classified as not irritating; test material did not cause corneal effects other than very slight corneal swelling; microscopic examinations of the corneas did not reveal any abnormalities	[5]
4-Amino-*m*-Cresol; purity not reported	none	undiluted; 50 mg	human reconstructed cornea	Eye irritation potential test using the human cornea model test in accordance with OECD TG 492; tissue exposed for 6 h; concurrent positive and negative controls utilized	Test system not suitable for assessment of test material; the test material led to a viability of 93.8%; however, the test material was proved to be a strong reducer of MTT reagent	[5]
			ANIMAL			
4-Amino-*m*-Cresol; purity not reported	50% propylene glycol	1.5%; 0.1 ml	5 female Pirbright white guinea pigs	Ocular irritation study; test material instilled in the conjunctival sac of the left eye; eye was not rinse; right eye served as control; ocular reactions recorded at 0.5, 1, 2, 3, 4, 6, and 7 h after instillation; further reading by fluoresce-instillation occurred at 24 and 48 h post-instillation	Minimal ocular irritation potential; conjunctival erythema observed in 1 animal without other macroscopic effects; no ocular irritation observed in remaining animals	[4]

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