Final Safety Assessment

2-Amino-4-Hydroxyethylaminoanisole and its Sulfate Salt as Used in Hair Dyes

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The 2011 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Christina Burnett, Scientific Analyst/Writer.

1101 17th Street, NW, Suite 412 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651 ♢ fax 202.331.0088 ♢ cirinfo@cir-safety.org

ABSTRACT

2-Amino-4-hydroxyethylaminoanisole and its salt, 2-amino-4-hydroxyethylaminoanisole sulfate are used as coupling agents in oxidative hair dyes. The CIR Expert Panel reviewed relevant animal and human data related to the ingredient. The Expert Panel concluded that 2-amino-4-hydroxyethylaminoanisole and 2-amino-4-hydroxyethylaminoanisole sulfate are safe for use in oxidative hair dye formulations. The Expert Panel cautioned that these ingredients should not be used in cosmetic products in which *N*-nitroso compounds may be formed.

INTRODUCTION

This report addresses the safety of 2-amino-4-hydroxyethylaminoanisole and its salt, 2-amino-4-hydroxyethylaminoanisole sulfate. Both of these cosmetic ingredients function as coupling agents in oxidative hair dyes.

CHEMISTRY

Definition and Structure

The definitions and structures of these 2 ingredients are presented in Table 1.

2-Amino-4-hydroxyethylaminoanisole and 2-amino-4-hydroxyethylaminoanisole sulfate are commonly used as components of oxidative hair dyes.[1] These ingredients act as "couplers" and react with "precursors." In a typical formulation, a precursor, such as p-phenylenediamine, is activated via an oxidant, such as hydrogen peroxide. The resultant activated, imino-iminium precursor can then proceed to couple with 2-amino-4-hydroxyethylaminoanisole or 2-amino-4-hydroxyethylaminoanisole sulfate to form a new compound (Figure 1). This in-situ coupled product is purported to be the actual dye that colors the hair in these types of oxidative hair dyes.

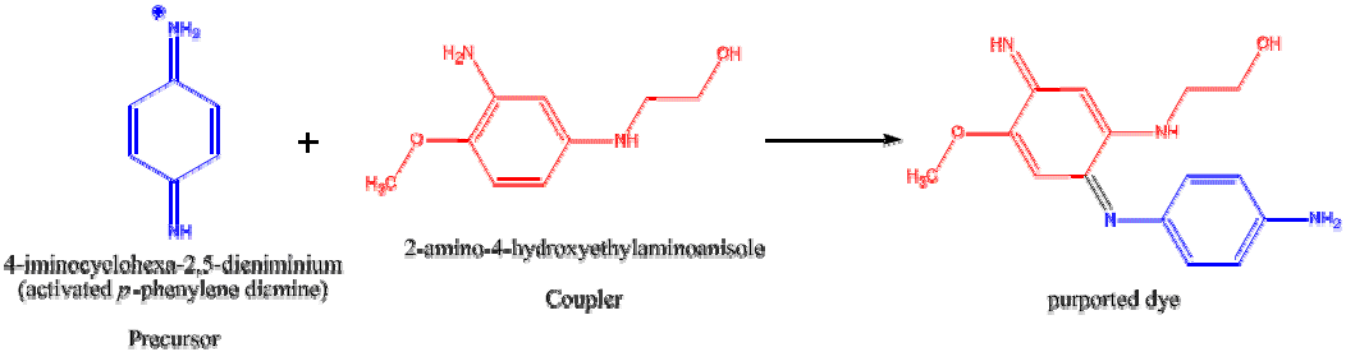


Figure 1. In-situ colorant formation.[1]

N-Nitrosation and Safety Issues

Although nitrosamine content has not been reported, 2-amino-4-hydroxyethylamino-anisole is a secondary amine, and potentially can be nitrosated. Of concern in cosmetics is the conversion (nitrosation) of secondary amines (R1-NH-R2), such as 2-amino-4-hydroxyethylamino-anisole (wherein R1 and R2 are ethanol and methoxyaniline), into *N*-nitrosamines that may be carcinogenic. Of the approximately 209 nitrosamines tested, 85% have been shown to produce cancer in laboratory animals.[2] Nitrosation can occur under physiologic conditions.[3] Depending on the nitrosating agent and the substrate, nitrosation can occur under acidic, neutral, or alkaline conditions. Atmospheric NO_2 may also participate in the nitrosation of amines in aqueous solution.[4] Accordingly, 2-amino-4-hydroxyethylamino-anisole and 2-amino-4-hydroxyethylaminoanisole sulfate should be formulated to avoid the formation of nitrosamines.

Physical and Chemical Properties

The available information on the physical and chemical properties of 2-amino-4-hydroxyethylaminoanisole and its sulfate salt are presented in Table 2.

Method of Manufacture

The manufacture of 2-amino-4-hydroxyethylaminoanisole sulfate can be accomplished via a six step synthesis from commercially available 4-methoxyaniline (Figure 2).[5,6] In the first step, 4-methoxyaniline is acetylated with acetic anhydride. The resultant acetamide is then nitrated with nitric and sulfuric acids. Then, *N*-(4-methoxy-3-nitrophenyl)acetamide is reduced from the amide to the amine, via reflux in hydrochloric acid. Next, the amine is oxidized to a carbamate using beta-chloroethyl chloroformate. Then, the resulting chloroethylcarbamate is heated with potassium hydroxide to produce the alcohol. Finally, the nitro group is reduced to the primary amine over Raney nickel and treated with sulfuric acid to produce the salt, 2-amino-4-hydroxyethylaminoanisole sulfate. The free base, 2-amino-4-hydroxyethylaminoanisole, easily can be prepared by neutralizing this salt.

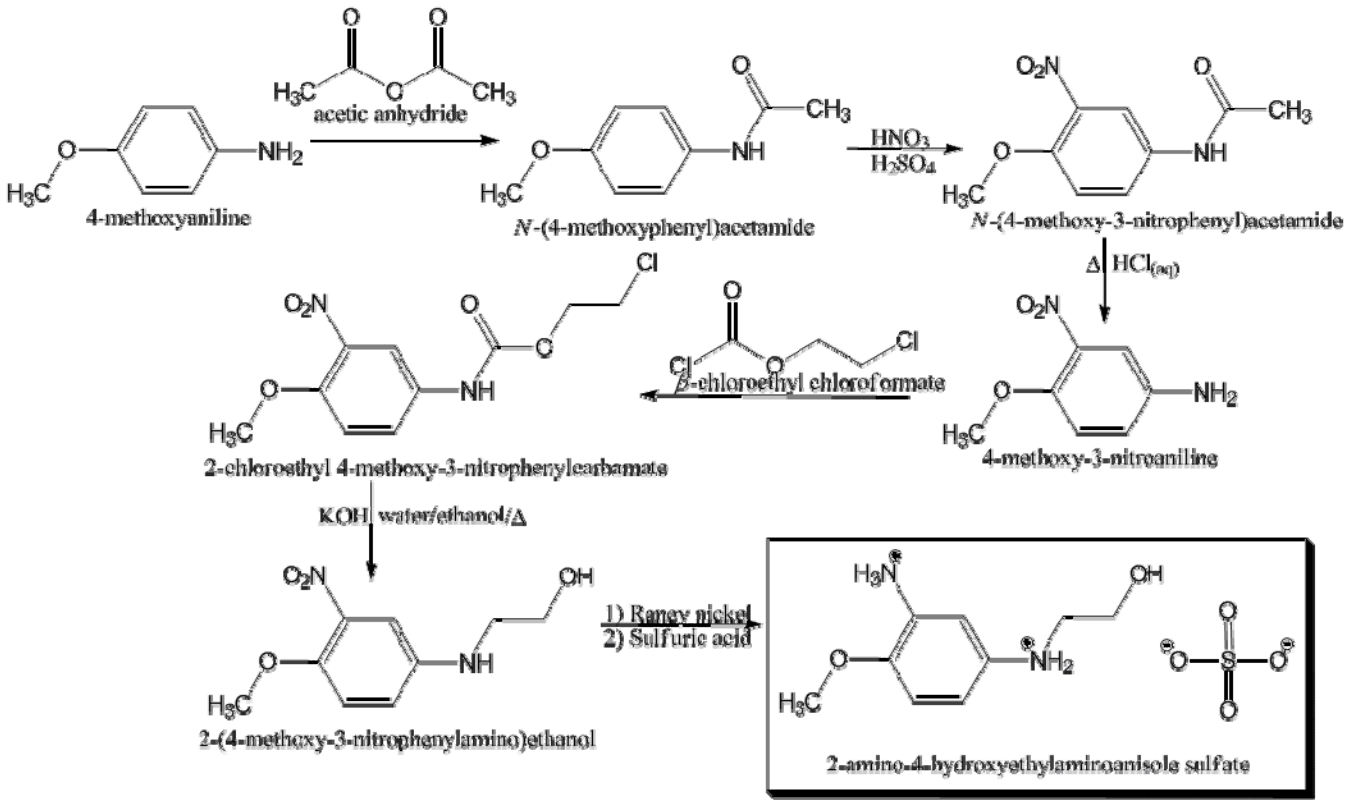


Figure 2. A method of synthesis for 2-amino-4-hydroxyethylaminoanisole sulfate.[5,6]

Impurities

Purity for 2-amino-4-hydroxyethylaminoanisole sulfate was reported to be 99.3-100% with HPLC at 210-304 nm.[7] The impurities 4-methoxyaniline (a starting material), 4-methoxy-3-nitroaniline (a synthesis intermediate), and 2-methoxy-5-nitroaniline (a by-product) were reported to be below the detection limit of 10 ppm, however, 2,4-diaminoanisole (a by-product) was reported at concentrations of 120-600 ppm in 4 different test batches. Methanol, ethanol, isopropanol, acetone, ethyl acetate, cyclohexane, methyl ethyl ketone, and monochlorobenzene (solvents used for the synthesis) were not detected at 100 ppm (detection limit).

USE

Cosmetic

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), 2-amino-4-hydroxyethylaminoanisole sulfate is used in a total of 94 hair coloring formulations.[8] In a survey of use concentrations conducted by the Personal Care Products Council, 2-amino-4-hydroxyethylaminoanisole sulfate is used at a concentration range of 0.008-1.5% (maximum 3% before dilution) in hair coloring products.[9] No uses or use concentrations were reported for 2-amino-4-hydroxyethylaminoanisole.

According to information provided by the Hair Coloring Technical Committee of the Personal Care Products Council, 2-amino-4-hydroxyethylaminoanisole sulfate is used as an oxidative hair coloring agent.[10] The intended maximum use ('on-head") concentration is 1.5%. This ingredient and a developer would be mixed at ratios between 1:1 to 1:3 (g dye:g hydrogen peroxide) during the hair dying process. It is general practice to apply 100 g of finished mixed hair dye product for approximately 30 minutes before rinsing off with water and shampoo and this process may be repeated on a monthly basis.

The Scientific Committee on Consumer Safety (SCCS) for the European Commission concluded that 2-amino-4-hydroxyethylaminoanisole sulfate would not pose a health risk to the consumer when used as an ingredient in oxidative hair dye formulations at a maximum concentration of 1.5%.[7] The SCCS could not exclude the possibility that this ingredient may be sensitizing. The Committee also determined that because 2-amino-4-hyroxyethylaminoanisole sulfate is a secondary amine, it should not be used with nitrosating substances and the nitrosamine content in the ingredient should be < 50 ppb.

Hair Dye Caution Statement - FDA labeling

These ingredients are considered coal tar hair dyes 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 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 a caution statement and patch test instructions for determining whether the product causes skin irritation. The CIR Expert Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 hours after application of the test material and prior to the use of a hair dye formulation.

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

Dermal/Percutaneous

In an in vitro percutaneous absorption study, [^{14}C] 2-amino-4-hydroxyethylaminoanisole sulfate at 1.5% as part of an oxidative hair dye formulation in the presence of hydrogen peroxide and a reaction partner was applied to excised, dermatomed pig skin.[11] The integrity of the skin was tested by measuring trans-epidermal water loss (TEWL) prior to test material application. The test substance (100 μg/cm^2) was applied to the skin samples for 30 minutes and then washed off with water and shampoo. Measurements for radioactivity in the receptor fluid were made at 16, 24, 40, 48, 64, and 72 h after application. At experiment end, the skin was heat-treated and the upper skin (stratum corneum + upper stratum germinativum) was mechanically separated from the lower skin (lower stratum germinativum + upper epidermis). Most of the test material was found in the rinsing solution (mean value ± SD = 1340.92 ± 50.57 μg/cm^2; 92.69%). The test material was also found in upper skin (2.352 ± 0.824 μg/cm^2; 0.162%), the lower skin (0.303 ± 0.219 μg/cm^2; 0.021%), and the fractions of receptor fluid collected over 72 h (0.409 ± 0.223 μg/cm^2; 0.028%). Total recovery of the radiolabeled 2-amino-4-hydroxyethylaminoanisole sulfate was 1446.71 ± 7.86 μg/cm^2 (96.59%). The total amount of radiolabeled 2-amino-4-hydroxyethylaminoanisole sulfate that was biologically available was 3.064 ± 0.88 μg/cm^2 (0.211%). The SCCS found that this study did not follow its requirements and performed a worst case scenario calculation that incorporated 2 standard deviations for dermal absorption of 2-amino-4-hydroxyethylaminoanisole sulfate, which yielded a value of 4.82 μg/cm^2.[7]

In an in vivo percutaneous absorption study, [^{14}C] 2-amino-4-hydroxyethylamino-anisole dihydrochloride was applied to the skin of Sprague-Dawley rats.[12] Groups of 3 male and 3 female rats received the test material at 0.75% in a commercial hair dye with hydrogen peroxide, 0.75% in a commercial hair dye without hydrogen peroxide, or in a 3.47% aqueous solution on a 9 cm^2 area. The mean dose for all 3 applications was 0.83 mg/cm^2 free base. Application time was 30 minutes, after which the formulations were scraped off and the test sites were rinsed with approximately 100 ml 3% shampoo solution and water. The areas were then covered to prevent the animals from licking the test sites during a 72 h observation period. Urine and feces were collected daily and the rats were killed 72 h after application. The application sites, blood, and several organs were collected and analyzed for radioactivity. After complete removal of the skin, the radioactivity in the carcass was measured.

The mean recovery rates for the free base at 0.75% in a commercial hair dye with hydrogen peroxide, 0.75% in a commercial hair dye without hydrogen peroxide, and in a 3.47% aqueous solution were 97.7%, 95.1%, and 99.9%, respectively. Most of the applied dose (93.6-98.9%) was recovered in the rinse solutions. At the cutaneous application site, 1.51%, 0.57%, and 0.75% of the applied formulation with hydrogen peroxide, without hydrogen peroxide, and in aqueous solution were recovered, respectively. Absorbed material was mainly excreted via the urine at 0.02%, 0.097%, and 0.180% of the applied formulation with hydrogen peroxide, without hydrogen peroxide, and in aqueous solution, respectively, and a very small amount was excreted in the feces: 0.008%, 0.027%, and 0.047%, respectively. The amount of test material in the carcasses and organs was close to or below the limit of detection for all 3 dose groups (0.005-0.008%). From these observations, it was determined that the absorption rates were 1.54% (12.8 μg/cm^2), 0.70% (5.8 μg/cm^2), and 0.99% (8.2 μg/cm^2) for the formulations with hydrogen peroxide, without hydrogen peroxide, and in aqueous solution. Using the highest absorption rate (12.8 μg/cm^2 as free base), the equivalent penetration rate for 2-amino-4-hydroxyethylaminoanisole sulfate is 19.7 μg/cm^2. This study was reported to not be GLP compliant.[7]

To the limit of detection, no parent compound or metabolites were found in the blood of 5 female volunteers after application by a professional hair dresser for 15 min. of 2-amino-4-hydroxyethylamino-anisole sulfate plus hydrogen peroxide oxidizing agent, containing 2.2% of the dye, indicating that less than 1.6 μg/cm² became bioavailable.[7]

Other Dose Administration

In an in vitro study, human intestinal epithelial (TC-7) cells were used to determine the bioavailability of 2-amino-4-hydroxyaminoanisole sulfate across the intestinal barrier.[14] Analysis of the donor (apical) and receiver (basolateral) samples was done using HLPC-MS/MS and the apparent permeability coefficient (P_{app}) was calculated for 2 independent experiments. ^{14}C-mannitol (~4 μM) 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. According to the laboratory's classification system, a $P_{app} < 2x10^{-6}$ cm/sec indicates low permeability. Ranitidine, which has a 50% absorption in humans, was used as a low permeability reference

compound, and it and another reference compound, propranolol, were well within the acceptance range and validated the study. The P_{app} for 2-amino-4-hydroxyethylaminoanisole sulfate was 73.3 x 10^{-6} cm/sec, which equates to a high permeability classification ($P_{app} \geq$ 20x 10^{-6} cm/sec) by this laboratory's classification system. It was concluded that 2-amino-4-hydroxyethylaminoanisole is readily absorbed in the gastrointestinal tract after oral administration.[7]

TOXICOLOGICAL STUDIES

Acute Toxicity

Oral – Non-Human

In an acute oral toxicity study, doses of 2-amino-4-hydroxyethylaminoanisole sulfate were administered to Wistar rats and CF 1 mice.[15] The dose groups for the rats (5 rats per sex per dose group) and mice (10 females per dose group) were 250, 375, 500, 625, and 750 mg/kg body weight. There was an additional dose group of 875 mg/kg body weight in the mice. Doses were established after a range finding study in mice found the median lethal dose to be less than 875 mg/kg body weight. The test material was administered once by oral gavage. Mortality and clinical signs of toxicity were recorded during the 14 day observation period. Body weights were recorded weekly. All animals were necropsied. Clinical signs of toxicity observed after dosing included tonic spasm, piloerection, and higher respiratory rate in both rats and mice. Three male and 2 female rats and 2 mice in the 375 mg/kg, 4 male and 2 female rats and 6 mice in the 500 mg/kg, all male and 4 female rats and 5 mice in the 625 mg/kg, and all male and female rats and 9 mice in the 750 mg/kg dose groups died between the first 24 h and 6 days after dosing. Additionally, all mice in the 875 mg/kg dose group died. No macroscopic changes were noted at necropsy. No data were provided regarding control groups. The LD_{50} of 2-amino-4-hydroxyethylaminoanisole sulfate in male rats, female rats, and female mice were calculated to be 475, 588, and 538 mg/kg body weight, respectively. This study was reported to not be GLP compliant.[7]

In another acute oral study, groups of 5 male and 5 female NMRI white mice received 125, 250, 500, 750, or 1000 mg/kg 2-amino-4-hydroxyethylaminoanisole sulfate by gavage.[16] Doses were established after a range finding study in mice found the median lethal dose to be less than 2000 mg/kg body weight. The test material was administered as a 1.25-10% dilution in deionized water. Mortality and clinical signs of toxicity were recorded during the 14 day observation period. Clinical signs of toxicity included those related to the central nervous system, coordination, reflexes, and autonomic functions with dose-dependent severity up to 72 h after administration. Weight gains were reduced in all surviving animals. One male and 1 female in the 125 mg/kg, 2 males and 1 female in the 250 mg/kg, 4 males and 3 females in the 500 mg/kg, 3 males and all females in the 750 mg/kg, and all males and 4 females in the 1000 mg/kg dose groups died within 24 to 72 h of dosing. No macroscopic changes were noted at necropsy. No data were provided regarding control groups. The calculated LD_{50} of 2-amino-4-hydroxyethylaminoanisole sulfate in male mice was 333 mg/kg body weight and in female mice was 351 mg/kg body weight. An LD_{50} for male and female mice combined was calculated to be 327 mg/kg body weight.

Repeated Dose Toxicity

Dermal – Non-Human

The dermal toxicity potential of 2-amino-4-hydroxyethylaminoanisole sulfate was evaluated in a 28 day study in SPF Pirbright White guinea pigs.[17] Dose groups of 5 animals of each sex and received 0, 50, 150, or 300 mg/kg body weight of the test material in tap water at a dose volume of 1 ml/kg body weight. The test material was applied once daily to a 3 x 4 cm area on clipped dorsal skin. The animals were checked twice daily for mortality. Clinical signs of toxicity were recorded daily and body weights were recorded weekly. Complete hematology and blood chemistry investigations and urinalysis were performed on day 0 and day 28. All animals were killed at the end of the treatment period. Select organs were weighed and a detailed necropsy was performed in all animals. Hearts and kidneys of the control and high dose group animals were studied histopathologically. Additionally, all gross lesions observed, the liver, and the skin of all dose groups were examined microscopically.

No deaths occurred and no relevant clinical signs were observed. Body weight gains were comparable to the control group. No treatment-related changes were observed in hematology, blood chemistry parameters, or urinalysis. No gross lesions were noted at necropsy and organ weights were comparable to the control group. The NOAEL for this 28-day dermal study of 2-amino-4-hydroxyethylaminoanisole sulfate was 300 mg/kg body weight.[17]

Oral – Non-Human

The potential for oral toxicity to 2-amino-4-hydroxyethylaminoanisole sulfate was investigated in Wistar HanBrl:WIST (SPF) rats.[18] Dose groups were comprised of 15 animals of each sex and received 0, 15, 50, or 200 mg/kg body weight of the test material in distilled water at a dose volume of 10 ml/kg body weight. The test material was administered by oral gavage once daily for 108/109 days. Clinical signs of toxicity, feed consumption, and body weights were recorded weekly. Ophthalmoscopic exams were performed before and after the treatment period. At week 15, functional observational battery, locomotor activity, and grip strength were tested, as were thyroid hormone levels (5 rats/sex/dose). Complete hematology and blood chemistry investigations and urinalysis were performed at the completion of the dosing period. All animals were killed at the end of the treatment period. Select organs were weighed and a detailed necropsy was performed in all animals. Histological exams were performed on organs and tissues of the control and high dose group animals, and on all gross lesions from all animals. Additionally, thyroids, spleens, kidneys, and pituitary glands were examined in the mid and low dose groups.

All animals survived until necropsy. Blue discoloration of the urine was noted in the female rats of the 15 mg/kg dose group as well as all animals in the 50 and 200 mg/kg dose groups. No clinical signs of toxicity were observed. No irregularities were observed during the functional observational battery or ophthalmoscopic exams. There were no treatment-related changes in feed consumption or body weight gains. Thyroid hormone levels were comparable to the control group.

In the 200 mg/kg body weight dose group, both sexes had slight anemia with compensatory reticulocytosis that presented as decreased red blood cell counts, decreased hemoglobin, increased methemoglobin, decreased hematocrit levels, and increased reticulocyte counts and maturity indices. Decreased creatinine, increased triglyceride sodium and chloride concentrations, slight proteinuria and increased bilirubin and nitrite was observed in both sexes of this dose group as well. Marginally increased thyroid-to-brain weight ratios were observed in both sexes and, in females, increased mean absolute and relative liver, kidney, and spleen weights were noted. Follicular cell enlargement of the thyroid gland was observed in both sexes of the 200 mg/kg dose group. Additionally, pigment storage and tubular swelling with necrosis of tubular cells and basal membrane thickening was observed in the kidneys, and increased mean grade of extra medullary hemopoiesis was observed in the spleen. Slight hypertrophy of chromophobic cells of the pituitary gland was seen in males of the 200 mg/kg dose group. Similar adverse effects were observed in the 50 mg/kg body weight dose group, but occurred mostly in the female rats. No adverse effects were noted in the hematology, blood chemistry, or gross necropsy of the 15 mg/kg body weight dose group. Due to the slight anemia and morphological and histological changes of the thyroid gland, kidneys, and pituitary gland in the 50 mg/kg dose group, the NOAEL was determined to be 15 mg/kg body weight of 2-amino-4-hydroxyethylaminoanisole sulfate in this 15 week rat study.[18]

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In a teratogenicity study, mated female Wistar HanBrl: WIST (SPF) rats received 2-amino-4-hydroxyethylaminoanisole sulfate by gavage on days 6-20 of gestation.[19] The doses used were based on the results of a range finding study. In the main study, groups of 22 rats received 0, 10, 30, or 150 mg/kg body weight of the test material in bi-distilled water. Maternal clinical signs were monitored twice daily. Body weights were recorded daily and food consumption was measured over 3-day periods. Dams were killed on gestation day 21. Complete necropsy and macroscopic examination of the organs was performed. Gravid uterus weights were determined and fetuses were removed, sexed, weighed, and examined externally. Implantation sites, resorption sites, and live and dead fetuses were recorded. Half of the fetuses were examined for soft-tissue abnormalities and half for skeletal abnormalities.

One animal in the high dose group was found dead on gestation day 10. The death was considered the result of a dosing error. No other treatment-related clinical signs of toxicity were observed. Urine of the 30 and 150 mg/kg dose groups was darkly discolored. A slight decrease in mean food consumption was observed for the entire treatment period in the 150 mg/kg dose group. This group also had slightly reduced body weight gain up to gestation day 16. No maternal treatment-related effects were observed at gross necropsy. There were also no treatment-related effects observed viscerally or skeletally in the fetuses. Uterus and placenta weights, number of corpora lutea, and implantations were similar to controls in all dose groups. Litter size, fetal mortality, fetal body weight, and sex ratio were also comparable to the controls. The maternal NOAEL was 30 mg/kg body weight and the fetal NOAEL was 150 mg/kg body weight in this rat teratology study.[19]

GENOTOXICITY

In Vitro

The mutagenic potential of 2-amino-4-hydroxyethylaminoanisole sulfate was studied in an Ames test using *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537, with and without S9 metabolic activation.[20] The test concentrations were 33, 100, 333, 1000, 2500, or 5000 µg/plate. The positive controls were 4-nitro-o-phenylenediamine, sodium azide, methyl methane sulfonate, and 2-aminoanthracene. Decreased background growth was observed at 2500 and 5000 µg/plate. No biologically relevant increases in revertant colony numbers were observed in any test strain at any dose level, with or without metabolic activation. Controls yielded expected results. It was concluded that 2-amino-4-hydroxyethylaminoanisole sulfate was not mutagenic in this assay.

The mutagenic activity of 2-amino-4-hydroxyethylaminoanisole sulfate was studied in mouse lymphoma L5178Y $TK^{+/-}$ cells at the *tk* locus.[21] After a range-finding test to measure cytotoxicity, an independent experiment was performed. The concentrations for the main experiment ranged from 0.5-100 µg/ml without S9 metabolic activation (precipitation was observed without S9 at $\geq$ 50 µg/ml) and 1.0-500 µg/ml (with S9 metabolic activation). The vehicle control was culture medium. The positive controls were benzo[α]pyrene with S9 and ethylmethanesulfonate without S9. The cultures were incubated with the test material for 4 h. Mutant frequency and cell survival were determined as was as the size/optical density of colonies and the ratio of small versus large colonies. A relative total growth of 20.61% compared to the control was observed in the 500 µg/ml with S9, while without S9, the relative total growth was 23.09% in 100 µg/ml. A biologically significant increase in the number of mutant colonies was observed with and without S9. With S9, the highest mutation factor was 2.32 at 500 µg/ml and the historical control range was exceeded at concentrations of 50 µg/ml and greater. Without S9, the highest mutation factor was 2.13 at 100 µg/ml. A biologically relevant shift towards small colonies indicating a clastogenic effect was observed following treatment with the test material, with and without metabolic activation. The

controls yielded expected results. It was concluded that 2-amino-4-hydroxyethylaminoanisole sulfate was mutagenic in this mouse lymphoma assay.

The genotoxic potential of 2-amino-4-hydroxyethylaminoanisole sulfate was studied in a micronucleus study using human peripheral blood lymphocytes.[22] The test material was tested at concentrations of 25, 100, and 150 µg/ml with S9 metabolic activation and at concentrations of 3, 5, and 8 µg/ml without S9 metabolic activation. The positive controls were cyclophosphamide with S9 and 4-nitroquinoline-1-oxide and vinblastine without S9. Cells were incubated with the test material 24 h after mitogen stimulation with phytohemagglutinin. Incubation for cells with metabolic activation was 3 h and 20 h for cells without metabolic activation. Cells were harvested 72 h after mitogen stimulation. The replication index (RI) was calculated from the proportions of mononucleate, binucleate, and multinucleate cells in 500 cells per replicate. One thousand binucleate cells from each culture were analyzed for the occurrence of micronuclei.

The RI at the highest concentration tested with and without metabolic activation were 63% and 68%, respectively. With S9, a concentration-related increase in the frequency of micronucleated binucleate (MNBN) cells was observed with statistical significance at 100 and 150 µg/ml. The frequency of MNBN also exceeded historical control range in single cultures in the same concentrations. Without S9, the frequencies of MNBN were similar to concurrent controls at all concentrations and within the historical range for vehicle controls. A small, borderline increase in the frequency of MNBN at 8 µg/ml was observed that exceeded historical vehicle controls in one culture only. The effect was considered equivocal due to the high level of cytotoxicity (68%) at this concentration. In this study, 2-amino-4-hydroxyethylaminoanisole sulfate was considered to be genotoxic.[22]

In Vivo

The genotoxic potential of 2-amino-4-hydroxyethylaminoanisole sulfate was studied in a micronucleus test using NMRI mice.[23] A dose range finding experiment preceded the main study. In the main study, groups of 5 mice of each sex received single intraperitoneal injections of 0, 20, 100, or 200 mg/kg body weight 2-amino-4-hydroxyethylaminoanisole sulfate in distilled water, with an additional group of mice receiving the 200 mg/kg dose. Control groups received distilled water or 40 mg/kg body weight cyclophophamide in 0.9% NaCl. Bone marrow cells were collected at 24 or 48 h (for the 200 mg/kg dose group). At least 2000 polychromatic erythrocytes per animal were analyzed, and the ratio between polychromatic and total erythrocytes per animal was determined by counting at least 200 immature polychromatic erythrocytes per animal. In the dose range finding study, toxic effects observed at 200 mg/kg included palpebral closure and lethargy within the first hour of treatment. Also in the range finding study, a dose of 400 mg/kg caused mortality, and signs of systemic toxicity were observed at both 400 mg/kg and 200 mg/kg. In the main study, no treatment-related mortalities or clinical signs of toxicity were observed. There was no statistically significant increase in micronuclei in the treatment groups when compared to the controls. The study concluded that up to 200 mg/kg body weight 2-amino-4-hydroxyethylaminoanisole sulfate was not genotoxic in this micronucleus assay.

The potential for 2-amino-4-hydroxyethylaminoanisole sulfate to induce unscheduled DNA synthesis (UDS) was assessed using male Wistar rats.[24] A dose range finding experiment preceded the main study. In the main study, groups of 5 rats received single oral doses of 75 or 750 mg/kg body weight 2-amino-4-hydroxyethylaminoanisole sulfate in bi-distilled water. Control groups received 10 ml/kg body weight bidistilled water or 100 mg/kg body weight 2-acetylaminofluorene. Rats were killed either 4 h after treatment (one group of the 750 mg/kg dose group) or 16 h after treatment (the 75 mg/kg dose group and the additional 750 mg/kg dose group). Liver perfusion was performed. At least 5 primary hepatocyte cultures were made from each animal and exposed for 4 h to 3H-thymidine. The dye-exclusion method was utilized to determine if any liver cell toxicity occurred. After the radiolabel exposure, the cells were washed and slides were prepared. At least 2 slides per animal were evaluated from the occurrence of UDS for 3 animals per dose group, which equated to 100 cells/animal. Heavily labeled S-phase cells were excluded from counting, and background grains were subtracted from the grains observed from the nucleus to obtain relevant net nuclear grains.

In the range finding study, mortality occurred at 1000 mg/kg within 24 h of dosing. Survivors in this group had reduced spontaneous activity, eyelid closure, and piloerection. At 750 mg/kg, there was no mortality. The kidneys, urine, and liver of the rats showed dark discoloration. In the main study, no mortality or clinical signs of toxicity were observed. No UDS induction was observed in the hepatocytes of the treated animals at any dose level or time period when compared to controls. There was no increase in the number of nuclear grains or the resulting net grains at either dose or time period. The study concluded that 2-amino-4-hydroxyethylaminoanisole sulfate did not induce DNA damage in this UDS assay.[24]

IRRITATION AND SENSITIZATION

Irritation

Dermal – Non-Human

In a dermal irritation study, 0.5 g of a commercial hair dye formulation that contained 3% 2-amino-4-hydroxyethylaminoanisole sulfate was applied to shaved skin (~ 6 cm^2) of 3 New Zealand White rabbits.[25] The test sites were then semi-occluded for 4 h, after which the test material was washed off with water. The skin was evaluated for reactions at 30 min, 1, 24, 48 and 72 h and then daily up to 14 days after the material was removed. Slight erythema (scores of 1 and 2) and very slight edema (score of 1) were recorded at several observation periods. These effects had completely disappeared within 7 days. In each rabbit, the mean 24/48/72 h scores for erythema and edema were 1.33 and 1.0; 1.67 and

1.0; and 1.33 and 1.0, respectively. The study concluded that 2-amino-4-hydroxyethylaminoanisole sulfate was a mild transient skin irritant when tested at 3%.

In another dermal irritation study, 1% 2-amino-4-hydroxyethylaminoanisole sulfate suspended in gum Arabic was applied to 10 female SPF white guinea pigs.[26] The test material was applied with a brush 3 times a day for 20 min durations on 2 consecutive days on the left and right clipped flanks of the animals. The treatment period was followed by a 3-day observation period. Any effects were scored according to the Draize method. Very slight erythema was observed in 2 of the animals on the second treatment day. No edema was observed. It was concluded that 2-amino-4-hydroxyethylaminoanisole sulfate at 1% was essentially non-irritating. This study was reported to not be GLP compliant.[7]

Ocular

A hair dye formulation containing 3% 2-amino-4-hydroxyethylaminoanisole sulfate was tested for ocular irritation potential in 3 New Zealand White rabbits.[27] The conjunctival sac of one eye of each rabbit was instilled with 0.1 ml of the test substance and not rinsed. The untreated eye served as a control. Both eyes were examined at 1, 24, 48, and 72 h post-treatment according to the Draize method. No cornea or iris effects were noted at any observation time. Slight conjunctival redness (score of 1) was noted at 24 h in all animals, but these effects were gone within 3 days of treatment. In each rabbit, the mean 24/48/72 h scores for conjunctival erythema were 0.67, 0.33, and 0.67, respectively. The study concluded that 3% 2-amino-4-hydroxyethylaminoanisole sulfate was a transient and mild irritant in the eyes of rabbits.

In another ocular irritation study, a 1% aqueous solution of 2-amino-4-hydroxyethylaminoanisole sulfate was tested in 10 female Pirbright White (SPF) guinea pigs.[28] The conjunctival sac of the right eye of the guinea pigs was instilled with 0.1 ml of the test substance and not rinsed. The left eye of the animals was left untreated and served as a control. At 24 h after application, the eyes were washed with fluorescein-sodium solution. Redness and discharge were observed in 5 of the guinea pigs 30 minutes after treatment. Redness was also observed in 2 animals at 7 h post-treatment, but this effect cleared at 24 h. No other ocular effects were observed. It was concluded that 1% 2-amino-4-hydroxyethylaminoanisole sulfate caused transient conjunctival irritation in this study. It should be noted that this study was reported to not be GLP compliant.[7]

Sensitization

Dermal – Non-Human

A local lymph node assay (LLNA) was performed using 2-amino-4-hydroxyethylaminoanisole sulfate dissolved in DMSO.[29] CBA/Ca female mice were divided into groups of 5 and received 0.25%, 0.5%, 1% or 2% of the test material on the ear surface (25 µl) once daily for 3 consecutive days. After each application, the ears were dried with a hair dryer for ~5 min. A positive control group received 0.25%, 0.5%, 1%, or 2% p-phenylenediamine in DMSO. Five days after the initial topical treatment, all animals were injected intravenously with 250 µl phosphate buffered saline containing 20 µCi of [^{3}H] methyl thymidine. Approximately 5 h after injection, the animals were killed and the auricular lymph nodes were excised. Single-cell suspensions were prepared from pooled lymph nodes, with the cells precipitated by trichloroacetic acid (TCA), and radioactivity measured by liquid scintillation. The stimulation indices (SI) were calculated.

No clinical signs of toxicity or deaths occurred during the treatment period in any dose group. The SI were1.29, 1.03, 1.12, and 1.42 for the 0.25%, 0.5%, 1%, and 2% dose groups, respectively. The estimated concentrations for a SI of 3 (EC_3) could not be calculated. The positive control group produced expected results and validated the study. The study concluded that 2-amino-4-hydroxyethylaminoanisole sulfate tested up to 2% in DMSO was not a skin sensitizer.[29]

QSAR

ATOPS-MODE quantitative structure-activity relationship (QSAR) model was utilized to predict the sensitization potential of all hair dye ingredients registered in Europe (229 substances as of 2004). [30] The model predicted 2-amino-4-hydroxyethylaminoanisole to be a weak sensitizer. The sensitization potential of the sulfate salt was not evaluated.

CLINICAL USE

Epidemiology

2-Amino-4-hydroxyethylaminoanisole and its sulfate salt are oxidative hair dye ingredients. While the safety of individual hair dye ingredients are not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. Currently available epidemiology studies provided insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers.

A detailed summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml.

RISK ASSESSMENT

The SCCS calculated the margin of safety for 2-amino-4-hydroxyethylaminoanisole sulfate to be 267.[7] To obtain this value, the systemic exposure dose (SED) of 0.056 mg/kg was determined based on the maximum absorption through the skin (4.82 µg/cm^2= the mean $\pm$ 2SD from an in vitro percutaneous absorption study), typical body weight (60 kg) and skin surface area (700 cm^2 for scalp surface area), and dermal absorption per treatment (3.374 mg, calculated using the values for maximum absorption and average body weight and skin surface area assumptions), which was then divided into the NOAEL of the 15 week rat (repeated dose oral toxicity) study (15 mg/kg).

SUMMARY

The cosmetic ingredients 2-amino-4-hydroxyethylaminoanisole and its salt, 2-amino-4-hydroxyethylaminoanisole sulfate, function as coupling agents in oxidative hair dyes. The free base currently has no reported uses by the FDA or the cosmetics industry. The sulfate salt is used in a total of 94 hair coloring formulations at a concentration range of 0.008-1.5%. 2-amino-4-hydroxyethylaminoanisole is not in current use.

In an in vitro percutaneous absorption study in dermatomed pig skin, 3.064 $\pm$ 0.88 $\mu g/cm^2$ (the mean $\pm$ SD; 0.211%) of 1.5% radiolabeled 2-amino-4-hydroxyethylaminoanisole sulfate in an oxidative hair dye formulation with a reaction partner was found to be biologically available. An in vivo study in rats found the equivalent penetration rate of radiolabeled 2-amino-4-hydroxyethylaminoanisole sulfate, which was calculated from the measured penetration rate of the free base, to be 19.7 $\mu g/cm^2$. A bioavailability study of 2-amino-4-hydroxyethylaminoanisole sulfate in human intestinal epithelial cells concluded that this chemical was readily absorbed in the gastrointestinal tract after oral administration.

An acute oral toxicity study of 2-amino-4-hydroxyethylaminoanisole sulfate determined LD_{50} values of 475 mg/kg and 588 mg/kg for male and female rats, respectively. The same study calculated a LD_{50} of 538 mg/kg in female mice. Clinical signs of toxicity observed after dosing included tonic spasm, piloerection, and higher respiratory rate in both rats and mice. In another acute oral toxicity study, the calculated total LD_{50} of 2-amino-4-hydroxyethylaminoanisole sulfate in male and female mice combined was 327 mg/kg.

The NOAEL for a 28-day dermal study of 2-amino-4-hydroxyethylaminoanisole sulfate in guinea pigs was 300 mg/kg body weight, which was the highest dose tested. In a 15-week oral study in rats, the NOAEL was determined to be 15 mg/kg body weight 2-amino-4-hydroxyethylaminoanisole sulfate. Slight anemia and morphological and histological changes to the thyroid gland, kidneys, and pituitary gland were observed at higher dose levels.

In a teratogenicity study where female rats received 2-amino-4-hydroxyethylaminoanisole sulfate by gavage at doses of 0, 10, 30, or 150 mg/kg body weight of the test material in bi-distilled water, the maternal NOAEL was 30 mg/kg body weight and the fetal NOAEL was 150 mg/kg body weight. Dams in the high dose group experienced effects on food consumption and body weight gains.

The ingredient 2-amino-4-hydroxyethylaminoanisole sulfate was not mutagenic in an Ames assay, but was found to be mutagenic in an in vitro mouse lymphoma assay and in a micronucleus study using human peripheral blood lymphocytes. In in vivo studies, 2-amino-4-hydroxyethylaminoanisole sulfate was not genotoxic in a mouse micronucleus test and it did not induce DNA damage in a UDS assay in rats.

In dermal irritation studies, 2-amino-4-hydroxyethylaminoanisole sulfate at 1% was essentially non-irritating to guinea pig skin; however, mild transient skin irritation was observed at 3% in rabbits. At these concentrations, 2-amino-4-hydroxyethylaminoanisole sulfate was a transient and mild irritant in the eyes of rabbits and guinea pigs. A local lymph node assay (LLNA) study concluded that 2-amino-4-hydroxyethylaminoanisole sulfate tested up to 2% in DMSO was not a skin sensitizer.

A QSAR model predicted 2-amino-4-hydroxyethylaminoanisole to be a weak sensitizer.

The most recent CIR review of available epidemiology studies concluded that there is insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers.

A margin of safety calculation for 2-amino-4-hydroxyethylaminoanisole sulfate by the EU's SCCS yielded a value of 267 compared to the NOAEL for repeated dose oral toxicity in rats.

DISCUSSION

The available data on 2-amino-4-hydroxyethylaminoanisole sulfate are sufficient; however, the Expert Panel noted gaps in the available safety data for the free base, 2-amino-4-hydroxyethylaminoanisole, in this safety assessment. The toxicological profile for the sulfate salt may be extended to the free base and, therefore, the data for the sulfate salt can be used to support the safety of both ingredients.

The Expert Panel recognized that 2-amino-4-hydroxyethylaminoanisole and its sulfate salt function as hair dye ingredients and that limited irritation and sensitization data are available. However, hair dyes containing these ingredients, as coal tar hair dye products, are exempt from certain adulteration and color additive provisions of the Federal Food, Drug, and Cosmetic Act, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Expert Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures.

While nitrosamine content of these hair dyes has not been reported, the Expert Panel noted these ingredients are secondary amines and potentially can be nitrosated. Accordingly, their use should be restricted to hair dye formulations to avoid formation of N-nitroso compounds.

In considering hair dye epidemiology data, the CIR Expert Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer or other toxicologic endpoints, based on lack of strength of the associations and inconsistency of findings.

The CIR Expert Panel noted that the use of oxidative hair dye formulations involves exposure to precursors and coupling agents as well as to their reaction products. Specifically, 2-amino-4-hydroxyethylaminoanisole sulfate is a coupler reacted with a precursor in the presence of an oxidizing agent to products the final dye product. While reaction intermediates may be formed, human exposure is to the precursors and coupling agents and to reaction products, no to reaction intermediates. The exposures to the precursors and couplers are low (they are consumed in the color forming reaction), and the exposures to reaction products are even lower (they are adsorbed into the hair shaft itself and physically retained there). Therefore, safety assessments of oxidative hair dyes are driven by the toxicological evaluation of the ingredients (i.e. precursors and coupling agents), more than by the reaction products formed during use, and not at all by reaction intermediates.

CONCLUSION

The CIR Expert Panel concluded that 2-amino-4-hydroxyethylaminoanisole and 2-amino-4-hydroxyethylaminoanisole sulfate are safe for use in oxidative hair dye formulations. Were the free base (2-amino-4-hydroxyethylaminoanisole) to be used in the future, the expectation is that it would be used at concentrations similar to the sulfate salt. The Expert Panel cautions that these ingredients should not be used in hair dye products in which *N*-nitroso compounds may be formed.

TABLES AND FIGURES

Table 1. Names, CAS Registry Numbers, Definitions, and Structures of the Diaminoanisole Ingredients.

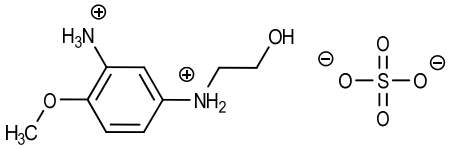
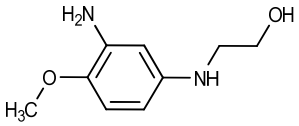
Ingredient CAS No.	Definition	Formula/structure
2-Amino-4-Hydroxyethylaminoanisole Sulfate 83763-48-8	2-Amino-4-Hydroxyethylaminoanisole Sulfate is sulfate salt of a 2,4-diamino-substituted aromatic, anisole, wherein the amine at the 4-position is substituted with ethanol.	
2-Amino-4-Hydroxyethylaminoanisole 83763-47-7	2-Amino-4-Hydroxyethylaminoanisole a 2,4-diamino-substituted aromatic, anisole, wherein the amine at the 4-position is substituted with ethanol.	

Table 2. Chemical properties

Property	Value	Reference
2-amino-4-hydroxyethylaminoanisole		
Molecular Weight g/mol	182.22	[31]
Molecular Volume cm^3/mol @ 20 ºC and 760 mmHg	149.1	[31]
Density g/ cm^3 @ 20 ºC and 760 mmHg	1.221	[31]
Vapor pressure mmHg@ 25 ºC	3.54E-7	[31]
Boiling Point ºC	401.8	[31]
logP @ 25 ºC	-0.916	[31]
Disassociation constant pKa @ 25 ºC	14.68	[31]
2-amino-4-hydroxyethylaminoanisole sulfate		
Physical Form	Powder	[7]
Color	Grey-blue	[7]
Molecular Weight g/mol	280.3	[7]
Density g/ cm^3 @ 20 ºC	1.541	[7]
Vapor pressure mmHg @ 20 ºC	1.5E-9	[7]
Melting Point ºC	138-149	[7,31]
Water Solubility g/L @ 20 ºC & pH 2.3	81.99	[7]
Acetone: Water Solubility g/L @ pH 2.1	>5	[7]
DMSO Solubility g/L	>90	[7]
Ethanol Solubility g/L	<10	[7]
logP @ 25 ºC pH 7.51	0.59	[7]

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