Safety Assessment of
1-Hydroxyethyl 4,5-Diamino Pyrazole Sulfate
as Used in Cosmetics

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All interested persons are provided 60 days from the above release date to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. Lillian Gill.

The 2016 Cosmetic Ingredient Review Expert Panel members are: Chairman, 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 Lillian J. Gill, DPA. This safety assessment was prepared by Christina L. Burnett, Scientific Analyst/Writer and Bart Heldreth, Ph.D., Chemist CIR.
ABSTRACT
The Cosmetic Ingredient Review Expert Panel (the Panel) reviewed the safety of 1-hydroxyethyl 4,5-diamino pyrazole sulfate, which functions as an oxidative hair dye ingredient. The Panel reviewed relevant animal and human data provided in this safety assessment, and concluded that 1-hydroxyethyl 4,5-diamino pyrazole sulfate is safe in the present practices of use and concentration in oxidative hair dye formulations.

INTRODUCTION
This report addresses the safety of 1-hydroxyethyl 4,5-diamino pyrazole sulfate. This cosmetic ingredient functions as a hair colorant.

CHEMISTRY
Definition and Structure
1-Hydroxyethyl 4,5-diamino pyrazole sulfate (CAS No. 155601-30-2) is the heterocyclic salt that conforms to the structure shown in Figure 1.

![Figure 1. 1-Hydroxyethyl 4,5-diamino pyrazole sulfate.](image)

1-Hydroxyethyl 4,5-diamino pyrazole sulfate is commonly used as a component of oxidative hair dyes. This ingredient acts as a “precursor” and reacts with a “coupler.” In a typical formulation, a precursor is activated via an oxidant, such as peroxide. The resultant activated precursor proceeds to react with a coupler to form in-situ a product that is purported to be the actual dye that colors hair in these types of oxidative hair dyes.

![Figure 2. Example of an oxidative hair dye coupling reaction](image)
Chemical and Physical Properties

1-Hydroxyethyl 4,5-diamino pyrazole sulfate is a low molecular weight (240 g/mol), water soluble powder. Physical and chemical properties of 1-hydroxyethyl 4,5-diamino pyrazole sulfate are found in Table 1.

Method of Manufacture

A number of processes can be found in the literature for the synthesis of 1-hydroxyethyl 4,5-diamino pyrazole sulfate. For example, in one method, 3,5-dibromo-4-nitropyrazole is added in an equimolar amount to sodium hydride, and then reacted with an equimolar amount of a hydroxyethylhalide. In a subsequent step, the now N-hydroxyethyl-substituted 3,5-dibromo-4-nitropyrazole is heated with benzyl amine to yield the 5-amino-3-bromo-4-nitropyrazole derivative. Catalyzed hydrogenation then reduces that intermediate to the final product, 1-hydroxyethyl 4,5-diamino pyrazole sulfate.

Impurities

The purity of 1-hydroxyethyl 4,5-diamino pyrazole has been reported to be 96.8% to 99.8%. Potential impurities in this hair dye ingredient are reagents and intermediate reaction products. These include 4-((5-amino-1(2-hydroxyethyl)-1H-pyrazol-4-yl)-imino)-4,5-dihydro-1-(2-hydroxyethyl)-5-imino-1H-pyrazole-sulfate (2:1; max. 0.145% w/w) and 1-methyl-4,5-diamino pyrazole sulfate (max. 0.7% w/w). Methanol, ethanol, isopropanol, n-propanol, acetone, ethylacetate, cyclohexane, methyl ethyl ketone, and chlorobenzene were not detected (detection limit 100 ppm for each).

Nitrosation

1-Hydroxyethyl 4,5-diamino pyrazole sulfate bears two primary aryl amine groups, plus two nitrogen atoms in the pyrazole ring. The concern in cosmetics is the conversion (nitrosation) of nitrogen-bearing ingredients into N-nitroso chemicals that may be carcinogenic. Of the approximately 209 nitrosamines tested, 85% have been shown to produce cancer in laboratory animals. Nitrosation can occur under physiologic conditions. Depending on the nitrosating agent and the substrate, nitrosation can occur under acidic, neutral, or alkaline conditions. Atmospheric NO₂ may also participate in nitrosation in aqueous solution. However, while many amines and amides are readily N-nitrosated to form isolatable nitrosamines and nitrosamides, aryl amines ultimately yield diazonium salts, instead of nitrosamines. And, while pyrazoles have been shown to undergo nitrosation, the reaction occurs on a carbon atom of the aromatic ring (i.e. not N-nitrosation), and does not result in the formation of N-nitrosamines. Thus, N-nitrosation of this ingredient is not expected to occur under use conditions.

USE

Cosmetic

The safety of the cosmetic ingredient included in this safety assessment is evaluated on the basis of the expected use in cosmetics. The Panel utilizes data received from the Food and Drug Administration (FDA) and the cosmetics industry in determining the safety of ingredients within that expected use. The data received from the FDA are those it collects from manufacturers on the use of individual ingredients in cosmetics by cosmetic product category in its Voluntary Cosmetic Registration Program (VCRP), and those from the cosmetic industry are submitted in response to a survey of the maximum reported use concentrations by category conducted by the Personal Care Products Council (Council).

According to 2015 VCRP data, 1-hydroxyethyl 4,5-diamino pyrazole sulfate is reported to be used in 105 formulations; 104 uses are in hair dyes and 1 use is in hair tint. The results of the concentration of use survey conducted in 2015 by the Council indicate 1-hydroxyethyl 4,5-diamino pyrazole sulfate is used at 0.71%-4.8% in hair dyes, where the product containing 4.8% is diluted to 2.4% before use.

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 United States’ Federal Food, Drug, and Cosmetic Act. In order to be exempt, the following caution statement must be displayed on all coal tar hair dye products:

Caution - this product contains ingredients which may cause skin irritation on certain individuals and a preliminary test according to accompanying directions should be made. This product must not be used for dyeing the eyelashes or eyebrows; to do so may cause blindness.
Product labels shall also bear patch test instructions for determining whether the product causes skin irritation. 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.

In 2012, a report was published regarding such self-testing for contact sensitization to hair dyes. These authors concluded that, in its present form, the hair dye self-test has severe limitations. The authors issued the warning that, if the use of a hair dye self-test to predict contact sensitization becomes widespread, there is severe risk that a tool has been marketed that may cause morbidity in European consumers. An accompanying editorial performed on behalf of the European Society of Contact Dermatitis (ESCD) asserted that industry is focusing on predicting the risks from exposure to hair dyes by having millions of European consumers perform a self-test prior to each hair dying and stated that it is the opinion of the ESCD that attention must be given to reducing the risks of serious allergic reactions by improving the safety of the products themselves.

Europe’s Scientific Committee on Consumer Safety (SCCS) issued an opinion on 1-hydroxyethyl 4,5-diamino pyrazole sulfate in 2011 with the conclusion that this ingredient in oxidative hair dye formulations with a maximum on-head concentration of 3.0% does not pose a risk to the health of the consumer, apart from its sensitizing potential. The European Commission has added 1-hydroxyethyl 4,5-diamino pyrazole sulfate to Annex III List of Substances Which Cosmetic Products Must Not Contain Except Subject to the Restrictions Laid Down, with the limitation that this ingredient must not exceed 3.0% when applied to the hair after mixing under oxidative conditions. The Commission has also placed the following labeling requirements on this ingredient and all other oxidative hair dye ingredients:

- The mixing ratio must be printed on the label. Hair colorants can cause severe allergic reactions. Read and follow instructions. This product is not intended for use on persons under the age of 16. Temporary “black henna” tattoos may increase your risk of allergy. Do not color your hair if you have a rash on your face or sensitive, irritated and damaged scalp, you have ever experienced any reaction after coloring your hair, or you have experienced a reaction to a temporary “black henna” tattoo in the past.

**TOXICOKINETICS**

**Absorption, Distribution, Metabolism, and Excretion**

**Oral/Dermal/Intravenous**

The toxicokinetics of 1-hydroxyethyl 4,5-diamino pyrazole sulfate was studied in female Wistar Kyoto rats. The animals received 14C-1-hydroxyethyl 4,5-diamino pyrazole sulfate through a single dermal application (30 min exposure, equivalent to 20 mg/kg body weight/day), oral gavage dose (10 or 250 mg/kg body weight), or intravenous (i.v.) administration (10 mg/kg body weight). There were 6 rats per group (4 groups total) for toxicokinetics studies, and 4 rats per group (4 groups total) for mass balance studies. Urine and feces were collected 24 h, and the animals were killed 120 h after dermal exposure. Urine and feces were collected from 0-8 h, 8-24 h, 24-48 h, 48-72 h, and 72-96 h after oral and i.v. exposure. The animals from the oral and i.v. exposure groups were killed 96 h after exposure. Total radioactivity was measured in urine and feces and in selected tissues and organs. The urine and feces samples of each group were pooled to determine the metabolite profile using high performance liquid chromatography (HPLC) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis methods. Blood was sampled from several rats per time point, 30 min and 1, 2, 4, 8, 24, 48, and 72 h after dermal exposure and 15 and 30 min and 1, 2, 4, 8, 24, and 48 h after oral and i.v. exposure. Total radioactivity and parent compound equivalent concentrations were determined.

Following dermal application, the mean cumulative recovery of radioactivity was 0.8% ± 0.5% of the applied dose in the urine and 0.8% ± 0.5% in the feces. Mean residual radioactivity in the carcass, tissues and blood was 2.4%, and the majority of this was recovered from the treated skin (1.7 ± 0.8%). Less than 0.1% of the total radioactivity was recovered in the cage wash. The mean mass balance was 91.1 ± 3.3%.

The radiolabeled test material was readily absorbed and rapidly excreted after oral administration. The mean cumulative recovery of radioactivity in the urine after 96 h was 73.3% ± 8.3% (low dose) and 75.7% ± 2.5% (high dose) of the applied dose. The mean cumulative recovery of radioactivity in the feces was 28.3% ± 2.3% (low dose) and 22.9% ± 1.0% (high dose) of the applied dose. Mean residual radioactivity in the carcass, tissues, and blood was 0.9% (low dose) and 0.6% (high dose) of the applied dose. Less than 5% of the total radioactivity was recovered in the cage wash. The mean mass balance was 107.7% ± 8.8% (low dose) and 101.3% ± 2.4% (high dose).
The mean percent recovery of radioactivity was 86.9% ± 8.0% in urine and 6.0% ± 2.6% in feces 96 h after i.v. administration. Mean residual radioactivity in the carcass, tissues, and blood was 0.9% of the dose. Less than 3% of the total radioactivity was recovered in the cage wash. The mean mass balance was 96.7% ± 4.6%.

Radioactivity in the urine of the animals dermally exposed to the test material was too low for accurate detection of metabolites. Five different metabolites each were isolated in the urine and feces following oral exposure to the radiolabeled test material, with the parent compound detected in the urine of high dose animals only. Only one major metabolite was common to the urine and feces. The metabolites detected, including oxidative and N-acetylated metabolites, were not clearly characterized because no standards had been identified for them. Inconsistent analytical results were obtained for the samples collected from the i.v. groups.

Oral absorption of the radiolabeled test material was fast, with maximum plasma concentrations reached 1 h after administration in both the low and high dose groups. Area under the curve \((AUC)_{0→∞}\) values were approximately 24 and 600 mg·h/kg for the low and high dose groups, respectively. The dose-normalized AUC values were 2.23 and 2.38, respectively. Absorption after dermal administration was faster than after oral administration; the maximum plasma concentrations were reached 30 min after dermal exposure. However, plasma concentrations remained low, leading to relatively low AUC values \((AUC_{0→∞} = 0.36 \text{ mg·h/kg and the corresponding } 0.03 \text{ dose-normalized value})\). In the plasma samples of the high oral dose group taken within 2 h of dosing, the radiolabeled test material was detected. Concentrations rapidly decreased with time, showing that the test material was quickly excreted. One metabolite peak was present in plasma samples following oral and i.v. administration, but nothing was detected after dermal application due to low radioactivity levels.

It was concluded in this study that dermal absorption of the radiolabeled 1-hydroxyethyl 4,5-diamino pyrazole sulfate was low (0.006 mg/cm², representing 2.4% of the applied dose). A worst case assumption of dermal bioavailability was calculated as 4% of the applied dose or 0.01 mg/cm². Excretion of dermally-absorbed material was mainly in the feces. Orally administered radiolabeled test material was extensively absorbed, readily distributed to all organs, extensively metabolized, and excreted via urine and feces. Oral absorption was between 78-83%.

**Dermal/Percutaneous Absorption**

In an in vitro percutaneous absorption study, 1-hydroxyethyl 4,5-diamino pyrazole sulfate in a hair dye formulation was applied to porcine back and flank skin in flow-through Teflon diffusion chambers. Approximately 400 mg aqueous cream formulation containing 3% of the test material was applied to a 4 cm² area of skin. The integrity of the skin was monitored at the beginning of the experiment using tritiated water. The experiment employed static and dynamic systems, with the dynamic system utilizing receptor solution pumped through the receptor chamber at a rate of 5 ml/h. Six chambers were used in each system. In both systems, the test material was removed after 30 minutes by washing the skin twice with 4 ml of water and then once with 4 ml of a shampoo formulation diluted to 16.7%, and then twice again with water. The washing solutions were combined and the amount of dye was determined by HPLC. Fractions of the receptor fluid were collected after 16, 24, 40, 48, 64, and 72 h, concentrated directly after the pump and analyzed immediately. At experiment end, the skin was heat-treated and the stratum corneum + upper stratum germinativum was mechanically separated from the lower stratum germinativum + upper dermis. Both skin compartments were extracted separately and the dye content was quantified with HPLC. The limit of quantification was 174 ng/HPLC-injection.

The integrity of each skin preparation was demonstrated by examining the penetration characteristics with tritiated water, with 1.2% to 1.7% of the applied dose found in the receptor fluids in the static system and 1.2% to 2.2% of the applied dose found in the dynamic system. The values were within the limit of acceptance (≤ 1.5%) for 4 of 6 samples in the static system and 1 of 6 samples in the dynamic system. Total recovery for the 5 samples was 87.7% ± 10.3%. Loss of 1-hydroxyethyl 4,5-diamino pyrazole sulfate was likely attributable to oxidation. The majority of the applied dose remained on the skin surface (87.5% ± 10.2%). At 72 h, 0.2 ± 0.3 µg/cm² was recovered in each the epidermis and upper dermis. After 72 h, 1-hydroxyethyl 4,5-diamino pyrazole sulfate in all fractions of the receptor fluid was below the limit of quantification. A maximum of 3.6 ± 5.7 µg/cm² could have passed through the skin during the 72 h permeation period, yielding an estimated total systemic exposure to 1-hydroxyethyl 4,5-diamino pyrazole sulfate of 3.8 ± 6.0 µg/cm² under use conditions.

In a similar percutaneous absorption study, 1-hydroxyethyl 4,5-diamino pyrazole sulfate at 3% in a hair dye formulation with hydrogen peroxide and a reaction partner was investigated using porcine skin. The skin preparations were continuously rinsed with physiological receptor fluid. Two independent experiments were performed with 6 diffusion cells per experiment. For calculations, the mean value of all valid skin samples (n=10) in contact with the test material was used. After checking skin integrity with tritiated water, 400 mg of the formulation was applied to the skin samples (dose area 100 mg/cm²) for 30 minutes and then washed off with water and shampoo. The determination of the amount of test material in the washings and in the receptor fluid was...
performed by measuring radioactivity with a scintillation counter. At termination of the experiment, the skin was heat-treated and the stratum corneum + upper stratum germinativum was mechanically separated from the lower stratum germinativum + upper dermis. Both skin compartments were extracted separately and the radioactivity was quantified.

Total recovery was 2817.46 ± 78.42 µg/cm² (97.7% ± 2.88%). After 60 minutes, 93.47% ± 2.84% of the test material was found in the rinsing solution. The stratum corneum + upper stratum germinativum contained 3.492 ± 0.906 µg/cm² (0.122% ± 0.031%) of the test material. The maximum amount of the test material determined to be biologically available was 1.163 ± 0.395 µg/cm² (0.040% of the applied dose).16

TOXICOLOGICAL STUDIES

Acute Toxicity

**Oral – Non-Human**

The acute oral toxicity of 1-hydroxyethyl 4,5-diamino pyrazole sulfate was tested in Sprague Dawley rats.17 Groups of 5 male and 5 female rats received a single dose of the test substance at 2000 mg/kg body weight in a volume of 10 ml/kg via gastric gavage. The vehicle was deionized water. The rats were observed daily for mortalities and clinical signs of toxicity for 14 days. Body weights were recorded on day 0, 7, and 14. No mortalities or any clinical signs of systemic toxicity were observed. Orange colored urine was observed in all animals. Body weight gain was within normal parameters. Large mesenteric lymph nodes and a grey-white covering on the spleen capsule were noted in 1 male. The acute oral LD₅₀ in this rat study was greater than the administered dose of 2000 mg/kg body weight.

**Inhalation – Non-Human**

Groups of 5 male and 5 female Wister rats were utilized in an acute inhalation study of 1-hydroxyethyl 4,5-diamino pyrazole sulfate.18 The animals were exposed for 4 h to an aerosol containing 5.24 ± 0.31g/m³ of the test material. The mass median aerodynamic diameter of the test particles was 3.3 ± 1.8 µm. No mortalities were observed. During exposure, slight to moderate decreased breathing frequency was observed. Discoloration of the fur was noted after exposure until necropsy. Half of the animals had grey discolored areas in the lungs at necropsy. The LC₅₀ for 1-hydroxyethyl 4,5-diamino pyrazole sulfate was greater than 5.24 g/m³.

Repeated Dose Toxicity

**Oral – Non-Human**

The potential for oral toxicity to 1-hydroxyethyl 4,5-diamino pyrazole sulfate was investigated in Sprague Dawley rats.19 Dose groups, comprised of 15 animals of each sex, received 0, 80, 250, or 800 mg/kg body weight of the test material in distilled water. The test material was administered by oral gavage once daily for 13 weeks. Clinical signs of toxicity and mortality were recorded daily, and feed consumption and body weights were recorded weekly. Ophthalmological exams were performed before the experiment and at week 12. Hematology and blood chemistry investigations, in addition to functional observational batteries (weeks 4, 8, and 12) and motor activity assessments (week 12-13), were also performed. All animals were killed at the end of the treatment period. Major organs were weighed and a detailed necropsy was performed in all animals. Select organs and tissues were collected and preserved from all animals, but only specimens from the control and high-dose groups were examined microscopically.

No effects related to the test material were observed in the low-dose animals. There was a slight but statistically significant decreased in body weight gain in females of the high-dose group. High-dose males had slight changes in red blood cell parameters (increase in mean corpuscular hemoglobin and in red blood cell volume) and an increase in relative spleen weight. No further details were provided. The no observed adverse effect level (NOAEL) was determined to be 250 mg/kg body weight/day.19

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

The potential reproductive toxicity of 1-hydroxyethyl 4,5-diamino pyrazole sulfate in distilled water was investigated in a one-generation study in Sprague-Dawley rats.20 Three groups of 24 male and 24 female rats received 0, 150, 300, or 900 mg/kg body weight of the test material via gavage prior to mating (9 weeks for males, 2 weeks for females), during mating, and during gestation and lactation (females only until postnatal day 21). The control group received the same dose volume of the vehicle. Clinical signs for toxicity and mortality were assessed daily. Weekly body weight measurements were made throughout the study in males and during the premating and mating periods in females. Pregnant and lactating females were weighed on gestation days 0, 6, 10, 15, and 20 and
on postnatal days 0, 4, 7, 14, and 21. Feed consumption was recorded weekly during premating and at 3-4 day intervals during gestation and lactation for females. Litter sizes were recorded and live pups were sexed, weighed, and examined for external anomalies. On postpartum day 4, litters were reduced to 8 pups and postnatal development of the offspring was monitored. All study animals were killed on postnatal day 21 and underwent gross necropsy. Testis, epididymides, and spleen weights were recorded. Histopathological examinations were performed on major reproductive system organs and spleen.

No treatment-related adverse effects on body weight, feed consumption or clinical signs were noted in the parental animals. Red staining of the skin/hair was noted in high dose animals, and violet staining was noted in the cage tray of all treated animals. Reproductive parameters, litter data, sex ratios, gestation, and pre-weaning development of the pups were not affected by treatment. Red staining on the dorsum was noted in pups of the mid- and high dose groups. At necropsy, a decrease in testes weight and an increase in spleen weight were noted in high dose parental animals when compared to controls. No treatment-related effects were observed during macroscopic or microscopic examinations. The NOAEL for parental animals was 300 mg/kg body weight/day and the fetal NOAEL was 900 mg/kg body weight/day.

In a developmental study, mated female Sprague-Dawley rats received 1-hydroxyethyl 4,5-diamino pyrazole sulfate by gavage on days 6-17 of gestation. Groups of 24 rats received 0, 100, 300, or 1000 mg/kg body weight of the test material in distilled water. Maternal clinical signs were monitored daily. Body weights were recorded on days 0, 2, 4, 6 through 17, and on day 20, and feed consumption was measured on day 20. Dams were killed on gestation day 20. Macroscopic inspections of major organs and tissues were performed during gross necropsy, and the number of corpora lutea, implantation sites, resorption sites, and live and dead fetuses were recorded. Viable fetuses were weighed, sexed, and examined for external defects and soft-tissue and skeletal abnormalities.

No mortalities were observed during the study. No treatment-related effects were observed in the low and mid-dose groups, with no indication of maternal toxicity noted at any dose level. Fetal survival during prenatal development was not affected at any dose level. In the high dose group, marginal adverse effects in terms of slightly delayed ossification, abnormal ossification patterns, and an increased incidence of fetuses with supernumerary ribs were observed. However, the distribution of sporadically observed malformations in all test groups and the controls did not indicate specific teratogenic effects of the test material. The maternal toxicity NOAEL was 1000 mg/kg body weight/day and the fetotoxicity NOAEL was 300 mg/kg body weight/day in this rat developmental study.

**GENOTOXICITY**

**In Vitro**

In an Ames test, the mutagenic potential of 1-hydroxyethyl 4,5-diamino pyrazole sulfate was studied in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538. The assay was performed with and without S9 metabolic activation at concentrations up to 5000 µg/plate. A reduction in revertant counts or sparse bacterial background lawn was observed in strains TA98 and TA1537 without S9 and in strains TA100 with S9 at 3000 µg/plate. No increase in revertant colony numbers was observed in any strain. Positive controls yield expected results. 1-Hydroxyethyl 4,5-diamino pyrazole sulfate was not mutagenic in this assay.

The genotoxic potential of 1-hydroxyethyl 4,5-diamino pyrazole sulfate was studied in a chromosome aberration study using human peripheral blood lymphocytes. In this 2-part study, the material was tested at 50, 150, and 500 µg/ml without S9 metabolic activation in experiment 1, and in experiment 2 at 500, 1500, and 5000 µg/ml, with and without S9. The incubation times for cells with metabolic activation were 24 h and 4 h without S9 in experiments 1 and 2, respectively, and 3.5 h for cells with S9 in experiment 2. Cytotoxicity was observed at the highest concentrations tested in both experiments, with and without S9. A statistically-significant and dose-dependent increase in the frequency of chromosomal aberrations was observed without S9, with a stronger effect observed after the 24-h treatment with 500 µg/ml than after the 4-h treatment with 5000 µg/ml. Only one test point yielded a positive response with S9. Positive controls yielded expected results. Under the conditions of this study, 1-hydroxyethyl 4,5-diamino pyrazole sulfate was considered clastogenic when tested in human lymphocytes without metabolic activation.

The potential of 1-hydroxyethyl 4,5-diamino pyrazole sulfate in deionized water to induce gene mutations at the HPRT locus was assessed using Chinese hamster V79 cells, with and without S9 metabolic activation. The assay was performed in 2 independent experiments, with the cells exposed to the test material for 4 h with and without metabolic activation in the first experiment and for 24 h without metabolic activation in the second experiment. In the first experiment, test concentrations were 0, 600, 900, 1200, 1800, or 2400 µg/ml with metabolic activation and 0, 40, 50, 60, 70, or 80 µg/ml without metabolic activation. In the second experiment, test concentrations were 0, 20, 25, 30, or 35 µg/ml without metabolic activation. No relevant or dose-dependent increase...
of mutation frequency was observed in either experiment, with an exception at 1200 µg/ml in the first experiment. At this dose an increase in mutant frequency was observed exceeding the threshold of 3 times the corresponding negative control. However, the absolute value of the mutant frequency was low and remained well within historical controls ranges, and the increase was not reproduced in the other culture. Thus, the increased mutant frequency was considered not biologically relevant. Positive controls yielded expected results. Under the conditions of this assay, 1-hydroxyethyl 4,5-diamino pyrazole sulfate was not mutagenic in this HPRT assay.

### In Vivo

The genotoxic potential of 1-hydroxyethyl 4,5-diamino pyrazole sulfate was studied in a micronucleus test using NMRI mice. Groups of 5 mice of each sex received single oral doses of 0, 500, 1000, or 2000 mg/kg body weight 1-hydroxyethyl 4,5-diamino pyrazole sulfate in 4% gum arabic. Bone marrow was collected at 24 or 48 h (high-dose group only) post-treatment. After treatment, all animals showed reduced motility, with greater effects observed at higher doses. In male mice, the highest dose tested led to a decrease in the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE). No increase in the incidence of micronucleated PCEs was induced by the test material. Positive controls yielded expected results. It was concluded that the test material was not clastogenic.

In a bone marrow chromosome aberration test, groups of 5 male and 5 female Wistar rats received intraperitoneal doses of 1-hydroxyethyl 4,5-diamino pyrazole sulfate in deionized water. The dose levels for the male rats were 0, 100, 200, or 400 mg/kg body weight, while the dose levels for the females were 0, 150, 300, or 600 mg/kg body weight. The rats received the test material in 2 intraperitoneal injections at 24 h apart. Bone marrow cells were collected 24 h after the final injection. One female rat died after receiving the high-dose. No bone marrow toxicity was observed as there was no relevant reduction of mitotic indices after treatment. No statistically significant increases in the incidence of chromosomal aberrations induced by the test material were observed. Positive controls yielded expected results. Under the conditions of this in vivo assay, 1-hydroxyethyl 4,5-diamino pyrazole sulfate was not clastogenic.

### CARCINOGENICITY

Although there are data regarding the relationship between hair dyes and carcinogenicity, no relevant published carcinogenicity studies on 1-hydroxyethyl 4,5-diamino pyrazole sulfate were identified in a literature search for this ingredient, and no unpublished data were submitted.

### IRRITATION AND SENSITIZATION

#### Dermal – Non-Human

The irritation potential of 1-hydroxyethyl 4,5-diamino pyrazole sulfate was assessed in 3 female New Zealand White albino rabbits. A semi-occlusive patch with approximately 0.5 g of the 99.8% pure test substance moistened with 0.5 ml deionized water was applied via a cellulose patch to an intact area (6 cm²) of shaved skin. The patches were removed after 4 h and remaining test substance was wiped off with a cellulose tissue. Erythema was observed in all 3 animals at 1 and 24 h post-patch removal, with the reaction continuing to the 48 h observation in 1 animal. Edema was noted in all 3 animals at 24 h post-patch removal. It was concluded that 1-hydroxyethyl 4,5-diamino pyrazole sulfate was irritating to rabbit skin.

#### Ocular

The ocular irritation potential of undiluted 1-hydroxyethyl 4,5-diamino pyrazole sulfate was tested in a 3 female New Zealand albino rabbits. Approximately 0.1 ml (95-100 mg) of the test material was instilled into the conjunctival sac of the right eye. The left eye served as a control. Conjunctival redness and edema up to grade 3 were observed in all animals 1, 24, 48, and 72 h after instillation. These effects persisted in 1 animal until day 21. Corneal opacity up to grade 2 was observed in all animals 1, 24, 48, and 72 h after instillation. Again, this effect persisted in 1 animal until day 21. Iridial reactions were noted in all animals up to 72 h after instillation, with reactions persisting in 1 animal until day 8. In this study, undiluted 1-hydroxyethyl 4,5-diamino pyrazole sulfate was considered to be very irritating to rabbit eyes.

A 5% solution of 1-hydroxyethyl 4,5-diamino pyrazole sulfate in deionized water was tested for ocular irritation in 3 female New Zealand albino rabbits following the same method described above. Conjunctival redness up to grade 2 was noted in all animals at 1 h after instillation, with the effect persisting until 48 h in 1
animal. Conjunctival edema was noted in 2/3 animals at 1 h. No other ocular reactions were observed. It was determined that a 5% solution of 1-hydroxyethyl 4,5-diamino pyrazole sulfate was irritating in rabbit eyes.

**Sensitization**

**Dermal – Non-Human**

The Buehler method was utilized to study the sensitization potential of 1-hydroxyethyl 4,5-diamino pyrazole sulfate in white petrolatum in female Hartley guinea pigs. There were 10 animals in the negative control group, 5 animals in the positive control group, and 20 animals in the test group. During the induction period, the test group received 40% of the test compound in petrolatum on clipped skin under an occlusive dressing on days 0, 7, 14, while the positive control group received 10% p-phenylenediamine in the vehicle and the negative control group received the vehicle alone. Each patch was applied for 6 h. The challenge patches were applied on day 28. Skin reactions were scored 24 and 48 h post-patch removal. Body weights were recorded on days 0 and 30. No positive reactions were observed in any of the animals in the test group. The positive control group yielded expected results. 1-Hydroxyethyl 4,5-diamino pyrazole sulfate was not a dermal sensitizer in this study.

The dermal sensitization potential of 1-hydroxyethyl 4,5-diamino pyrazole sulfate was evaluated in a Magnusson-Kligman guinea pig maximization study. The test group consisted of 10 female Hartley guinea pigs, and the negative control group had 5 animals. A concentration of 1% in physiological saline was used for the intradermal induction, 40% w/w in white petrolatum for dermal induction, and 40% w/w in petrolatum for the dermal challenge patch. The patches (2 x 4 cm) in the dermal induction and challenge were occluded. Skin was examined for reactions 24 and 48 h after removal of the challenge patch. No reactions were observed in the control animals. In the treated animals, a positive response was observed in all animals at both observation periods. Histopathological examination revealed hyper- and parakeratosis, vesicle formation, and lymphohistiocytic infiltration along with other skin reactions (no further details provided). Due to the 100% sensitization rate, it was determined that 1-hydroxyethyl 4,5-diamino pyrazole sulfate was an extremely potent contact allergen in this maximization study.

The contact hypersensitivity of 1-hydroxyethyl 4,5-diamino pyrazole sulfate was assessed in a local lymph node assay (LLNA). CBA female mice were divided into groups of 5 and received 0%, 0.5%, 1.5%, 5%, or 10% of the test material in either acetone/water (1:1, v/v) mixed with olive oil (4:1) or dimethylsulfoxide (DMSO) on the ear surface (25 μl/ear) once daily for 3 consecutive days. A positive control group received 1% p-phenylenediamine in DMSO. Clinical signs of toxicity were assessed daily and body weights were measured on days 1 and 5. On day 5, all animals were injected intravenously with 20μCi [3H] methyl thymidine and the proliferation of lymphocytes in the draining lymph nodes was measured. The stimulation indices (SI) were calculated.

No clinical signs of toxicity, deaths, or skin reactions occurred during the treatment period in any dose group. In acetone/water/olive oil, the test material was negative, as there was less than a 3-fold increase in isotope incorporation in the draining auricular lymph nodes relative to the vehicle. The mean SI were 0.9, 1.0, 0.8, and 1.0 at concentrations of 0.5%, 1.5%, 5.0%, and 10.0%, respectively. In DMSO, the test material was also negative. The mean SI were 1.4, 1.7, 1.5, and 1.8 at concentrations of 0.5%, 1.5%, 5.0%, and 10.0%, respectively. An EC3 value (concentration inducing a stimulation index of 3) was not calculated. The positive control yielded expected results. It was concluded that 1-hydroxyethyl 4,5-diamino pyrazole sulfate was not a skin sensitizer in this LLNA.

**QSAR**

A 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). The model predicted 1-hydroxyethyl 4,5-diamino pyrazole sulfate to be a moderate to strong sensitizer. The QSAR analysis involved calculating TOPological Substructural MOlecular DEsign (TOPS-MODE) descriptors and correlating them to unspecified sensitization data from LLNAs that were available in July 2003.

**Epidemiology**

1-Hydroxyethyl 4,5-diamino pyrazole sulfate is used as a precursor in oxidative hair dyes. 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/cir-findings](http://www.cir-safety.org/cir-findings).
SUMMARY

1-Hydroxyethyl 4,5-diamino pyrazole sulfate is used as a precursor in oxidative hair dyes. According to 2015 VCRP data and the results of an industry survey of concentration of use, 1-hydroxyethyl 4,5-diamino pyrazole sulfate is used in a total of 105 hair coloring formulations at maximum concentrations of 0.71%-4.8% in hair dyes, where the product containing 4.8% is diluted to 2.4% before use.

In a toxicokinetics study, radiolabeled 1-hydroxyethyl 4,5-diamino pyrazole sulfate was extensively absorbed, readily distributed into all organs, extensively metabolized, and excreted via urine and feces following oral administration. Oral absorption was between 78%-83%. Dermal absorption of the radiolabeled material was low (0.006 mg/cm², representing 2.4% of the applied dose), and blood plasma concentration peaked within 30 min after application to the skin. Excretion of dermally-absorbed material was mainly in the feces. In in vitro percutaneous absorption studies using pig skin, the total recovery was 87.7% ± 10.3% of 1-hydroxyethyl 4,5-diamino pyrazole sulfate in formulation without hydrogen peroxide and 97.7% ± 2.88% of the test material with hydrogen peroxide. A total systemic exposure to the test material was determined to be about 3.8 ± 6.0 µg/cm² without hydrogen peroxide and 1.163 ± 0.395 µg/cm² with hydrogen peroxide under use conditions.

In rats, the acute oral LD₅₀ of 1-hydroxyethyl 4,5-diamino pyrazole sulfate was greater than 2000 mg/kg body weight, and the acute inhalation LC₅₀ was greater than 5.24 g/m³.

In a 13-week repeated dose study in rats that received 0, 80, 250, or 800 mg/kg body weight of the test material in distilled water, the NOAEL was determined to be 250 mg/kg body weight/day. No effects related to the test material were observed in the low-dose animals. There was a slight but statistically significant decrease in body weight gain in females of the high-dose group. High-dose males had slight changes in red blood cell parameters (increase in mean corpuscular hemoglobin and in red blood cell volume) and an increase in relative spleen weight.

The NOAEL for parental Sprague-Dawley rats was 300 mg/kg body weight/day and the fetal NOAEL was 900 mg/kg body weight/day in an oral reproductive toxicity study of 1-hydroxyethyl 4,5-diamino pyrazole sulfate in distilled water tested at 0, 150, 300, or 900 mg/kg body weight. At necropsy, a decrease in testes weight and an increase in spleen weight were noted in high dose parental animals when compared to controls. No other adverse treatment-related effects were observed. In a developmental study in rats, the maternal toxicity NOAEL was 1000 mg/kg body weight/day and the fetotoxicity NOAEL was 300 mg/kg body weight/day. Rats received 0, 100, 300, or 1000 mg/kg body weight of 1-hydroxyethyl 4,5-diamino pyrazole sulfate in distilled water. In the high dose group, marginal adverse effects in terms of slightly delayed ossification, abnormal ossification patterns, and an increased incidence of fetuses with supernumerary ribs were observed. However, the distribution of sporadically observed malformations in all test groups and the controls did not indicate specific teratogenic effects of the test material.

1-Hydroxyethyl 4,5-diamino pyrazole sulfate was not mutagenic in an Ames assay at concentrations up to 5000 µg/plate or in a HRPT locus assay in V79 cells at up to 2400 µg/ml, but was considered clastogenic when tested in human lymphocytes without metabolic activation at concentrations up to 500 µg/ml. Cytotoxicity was observed at the highest concentrations tested in the human lymphocytes, though, and the test material was not clastogenic with metabolic activation. In vivo micronucleus (mice) and bone marrow chromosome aberration (rats) studies indicated that 1-hydroxyethyl 4,5-diamino pyrazole sulfate was not clastogenic.

In dermal and ocular irritation studies, 1-hydroxyethyl 4,5-diamino pyrazole sulfate was irritating to rabbit skin (0.5 g of a 99.8% pure sample) and was classified as irritating to very irritating in rabbit eyes (5% and undiluted, respectively).

1-Hydroxyethyl 4,5-diamino pyrazole sulfate was not a dermal sensitizer in a Buehler sensitization study in guinea pigs at 40% in white petrolatum or in an LLNA study up to 10%, but was determined to be an extremely potent contact allergen in a Magnusson-Kligman guinea pig maximization study when tested at the same concentration in the same vehicle. A QSAR model predicted 1-hydroxyethyl 4,5-diamino pyrazole sulfate to be a moderate to strong sensitizer.

Currently available epidemiology studies provided insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers.

Although there are data regarding the relationship between hair dyes and carcinogenicity; no relevant published carcinogenicity studies on 1-hydroxyethyl 4,5-diamino pyrazole sulfate were identified in a literature search for this ingredient, and no unpublished data were submitted.

DISCUSSION

The Panel reviewed the findings of safety test and toxicokinetic studies that included dermal and ocular irritation, sensitization, and absorption potential of 1-hydroxyethyl 4,5-diamino pyrazole sulfate. The Panel noted that carcinogenicity data were not available; however, the majority of the results of in vitro and in vivo genotoxicity tests were negative. Additionally, the available data on acute and repeated dose toxicity, reproductive and
developmental toxicity, irritation and sensitization for 1-hydroxyethyl 4,5-diamino pyrazole sulfate indicate there is a low potential for this oxidative hair dye ingredient to cause systemic effects, thus the Panel was able to conclude this ingredient is safe for use in hair dyes.

While 1-hydroxyethyl 4,5-diamino pyrazole sulfate bears two primary aryl amine groups, and two nitrogen atoms in the pyrazole ring, N-nitrosation of this ingredient is not expected to occur. Many amines and amides are readily N-nitrosated to form isolatable nitrosamines and nitrosamides, but aryl amines ultimately yield diazonium salts, instead of nitrosamines. And, while pyrazoles have been shown to undergo nitrosation, the reaction occurs on a carbon atom of the aromatic ring (i.e. not N-nitrosation but C-nitrosation), and does not result in the formation of N-nitrosamines. Accordingly, the concern in cosmetics of the conversion (nitrosation) of nitrogen-bearing ingredients into N-nitroso chemicals that may be carcinogenic is not relevant to the ingredient in this report.

The Panel recognized that 1-hydroxyethyl 4,5-diamino pyrazole sulfate functions as a hair dye ingredient and that hair dyes containing this ingredient, as coal tar hair dye products, are exempt from certain adulteration and color additive provisions of the Federal Food, Drug, and Cosmetic Act, 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. 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 toxicologic endpoints, based on lack of strength of the associations and inconsistency of findings.

The Panel noted that the use of oxidative hair dye formulations involves exposure to precursors and coupling agents as well as to their reaction products. While reaction intermediates may be formed, human exposure is to the precursors and coupling agents and to reaction products, not 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 onto the hair shaft itself and physically retained there). Therefore, it was the consensus of the Panel that safety assessments of oxidative hair dyes are primarily determined by the toxicological evaluation of the ingredients (i.e. precursors and coupling agents), rather than by the reaction intermediates or products formed during use.

CONCLUSION

The CIR Expert Panel concluded 1-hydroxyethyl 4,5-diamino pyrazole sulfate is safe as an oxidative hair dye ingredient in the present practices of use and concentration.
<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
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<tr>
<td>Physical form</td>
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<td>Molecular weight g/mol</td>
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REFERENCES


