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

Status: Scientific Literature Review for Public Comment
Release Date: July 30, 2015
Panel Meeting Date: December 14-15, 2015

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date to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a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 2015 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.

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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 1.¹

![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.² 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.² 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.⁷ 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**

Potential impurities in 1-hydroxyethyl-4,5-diamino pyrazole sulfate 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**

Information concerning the propensity of 1-hydroxyethyl-4,5-diamino pyrazole sulfate to form nitrosamines was neither found in the literature nor made available, nor was data concerning such reactivity expected. 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 NO$_2$ 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 Cosmetic Ingredient Review Expert Panel (Panel) utilizes data received from the Food and Drug Administration (FDA) and the cosmetics industry in determining the expected cosmetic 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 the 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.

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 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.

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.13

Europe’s Scientific Committee on Consumer Products (SCCP) found the available data to be inadequate to assess the safety of 1-hydroxyethyl-4,5-diamino pyrazole sulfate. Data needs included in vitro mammalian cell genotoxicity tests and in vitro percutaneous absorption and genotoxicity studies performed under the SCCP’s guidance for hair dyes.2 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.10 The Commission has also placed the following labeling requirements on this ingredient and all other 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.11

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.2 The animals received the radiolabeled test material 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 (another 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 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).

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) 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 mg•h/kg and the corresponding 0.03 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 metabolized. 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%.

**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. 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 LD_{50} 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. The animals were exposed for 4 h to an aerosol containing 5.24 \pm 0.31 \text{g/m}^3 of the test material. The mass median aerodynamic diameter of the test particles was 3.3 \pm 1.8 \text{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 on the lungs at necropsy. The LC_{50} for 1-hydroxyethyl-4,5-diamino pyrazole sulfate was greater than 5.24 g/m^{3}.

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. Dose groups were comprised of 15 animals of each sex and 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. Ophthalmoscopic 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 12-3), 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 histopathologically.

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.

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. Three groups of 24 male and 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, weighted, 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 histopathologic 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 inspection of major organs and tissues were performed during gross necropsy, and number of corpora lutea, implantation sites, resorption sites, and live and dead fetuses were recorded.
Viable fetuses were weighed, sexed, and examined for gross, 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.2

**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 TA1538.2 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 test material was tested at 50, 150, and 500 μg/ml 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. 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, as well as 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.

**In Vivo**

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.2 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**

No relevant published carcinogenicity studies on 1-hydroxyethyl-4,5-diamino pyrazole sulfate were identified in a literature search for these ingredients, and no unpublished data were submitted.
IRRITATION AND SENSITIZATION

Irritation

**Dermal – Non-Human**

The irritation potential of 1-hydroxyethyl-4,5-diamino pyrazole sulfate was assessed in 3 female New Zealand White albino rabbits.² 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 3 female New Zealand albino rabbits.² 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.² 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 to 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.² 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.² 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.

**QSAR**

A non-validated 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).¹⁴ 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.circ-safety.org/cir-findings](http://www.circ-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.

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 dermal absorbed material was mainly in the feces. In an in vitro percutaneous absorption study using pig skin, 1-hydroxyethyl-4,5-diamino pyrazole sulfate the total recovery was 87.7% ± 10.3%. The majority of the applied dose remained on the skin surface (87.5% ± 10.2%). A total systemic exposure to the test material was determined to be about 3.8 ± 6.0 µg/cm² 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 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.

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 indicated 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, but was considered clastogenic when tested in human lymphocytes without metabolic activation at concentrations up to 500 µg/mL. 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, 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.

No relevant published carcinogenicity studies on 1-hydroxyethyl-4,5-diamino pyrazole sulfate were identified in a literature search for these ingredients and no unpublished data were submitted.
DATA NEEDS

CIR is seeking further toxicological data that would help the CIR Expert Panel assess the safety of the use of these ingredients in cosmetics, especially from dermal or ocular exposures to ingredients in formulation at maximal use concentrations.
### Table 1. Physical and chemical properties of 1-hydroxyethyl-4,5-diamino pyrazole sulfate.

<table>
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<tr>
<th>Property</th>
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<tr>
<td>Physical form</td>
<td>White to light pink powder</td>
<td>2</td>
</tr>
<tr>
<td>Molecular weight g/mol</td>
<td>240.24</td>
<td>2</td>
</tr>
<tr>
<td>Melting point °C</td>
<td>174.7</td>
<td>2</td>
</tr>
<tr>
<td>log P at 30 °C, pH 7.0</td>
<td>-1.75</td>
<td>2</td>
</tr>
<tr>
<td>Vapor pressure hPa at 20 °C</td>
<td>1.65 x 10^-8</td>
<td>2</td>
</tr>
<tr>
<td>Density at 20 °C</td>
<td>1.87</td>
<td>2</td>
</tr>
<tr>
<td>Solubility in water at 20 °C (% g/l)</td>
<td>666</td>
<td>2</td>
</tr>
<tr>
<td>Solubility in water/acetone and DMSO (%)</td>
<td>≥ 10</td>
<td>2</td>
</tr>
<tr>
<td>Purity titer as determined by HPLC (%)</td>
<td>96.8-99.8</td>
<td>2</td>
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<tr>
<td>Ash content (%)</td>
<td>&lt; 2</td>
<td>2</td>
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REFERENCES


