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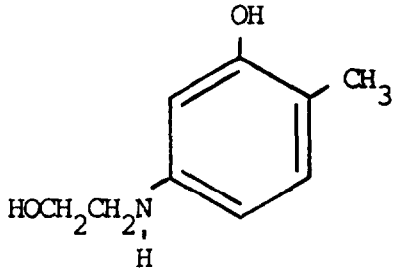
Final Report on the Safety Assessment of 2-Methyl-5-Hydroxyethylaminophenol

2-Methyl-5-Hydroxyethylaminophenol is used in oxidative hair dyes as a coupler at concentrations ranging from ≤ 0.1 to 5.0%. Only slight absorption was observed in skin studies. The LD_{50} of the ingredient in mice ranged from 2.5 to 3.84 g/kg. The ingredient was less of an irritant when tested alone than when tested in hair dye formulations. The compound is neither a mutagen nor a teratogen. 2-Methyl-5-Hydroxyethylaminophenol was classified as a nonirritant and weak sensitizer in human studies. Precautionary statements and instructions for patch testing are required on the label when used in oxidative hair dyes. On the basis of the available data included in the report, 2-Methyl-5-Hydroxyethylaminophenol is considered to be safe for use in the present practices of use and concentrations.

CHEMISTRY

Chemical and Physical Properties

2-Methyl-5-Hydroxyethylaminophenol (CAS No. 55302-96-0) is a substituted aromatic amine with the following structure:[1]



2-Methyl-5-Hydroxyethylaminophenol

Other chemical names include: 5-[(2-hydroxyethyl)amino]-2-methylphenol, 1-methyl-2-hydroxy-4-(β-hydroxyethyl)aminobenzene, 2-hydroxy-4-(β-hydroxyethyl)aminotoluene, 6-methyl-3-β-hydroxyethylaminophenol, and phenol, 5-[(2-hydroxy-

ethyl)amino)-2-methyl.[1,2] Chemical and physical properties of 2-Methyl-5-Hydroxyethylaminophenol are listed in Table 1.

Methods of Production

Information concerning the method of manufacture of 2-Methyl-5-Hydroxyethylaminophenol has not been found. Generally, aminophenols are manufactured via the reduction of nitrophenols.[4]

Reactivity

Aminophenols undergo reactions that are characteristic of phenols and aromatic amines, respectively. Typical reactions of phenols include the formation of esters and ethers; those of aromatic amines include the formation of amides.[5,6]

Analytical Methods

The commercial grade of 2-Methyl-5-Hydroxyethylaminophenol used in cosmetics has been identified via high-performance liquid chromatography and high-performance thin-layer chromatography.[7] Ultraviolet spectral analysis of the compound indicates absorption maxima at 207, 242, and 295 nm.[8]

IMPURITIES

2-Methyl-5-Hydroxyethylaminophenol is 98.9% pure.[2] As determined by high-performance liquid chromatography, it contains 0.07% 1-methyl-2-hydroxy-4-aminobenzene. It also contains less than or equal to 1.0% sodium chloride, as determined by potentiometric titration of sodium chloride with 0.1 N silver nitrate.[9]

USE

Cosmetic Use

Commercial 2-Methyl-5-Hydroxyethylaminophenol is a hair dye coupler used in oxidation hair dye formulations at a maximum concentration of 2%. In combination with hydrogen peroxide, the use concentration upon application is 1.0%.[2,10]

The cosmetic formulation listing which is made available by the Food and Drug Administration (FDA) is compiled through voluntary filing of such data in accordance

TABLE 1. Properties of 2-Methyl-5-Hydroxyethylaminophenol

		References
Molecular weight	167	2
Form	Light to dark beige powder	3
Odor	None	3
Melting point	93.5°C	2
Solubility	Soluble in distilled water, ethanol (96% by volume), and acetone	3

with Title 21 part 720.4 of the Code of Federal Regulations.[11] Ingredients are listed in prescribed concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a 2- to 10-fold error in the assumed ingredient concentration. 2-Methyl-5-Hydroxyethylaminophenol is present in 54 hair dyes and colors (all types requiring caution statement and patch test) at concentrations ranging from ≤ 0.1 to 5% (Table 2).[12]

Hair coloring formulations containing 2-Methyl-5-Hydroxyethylaminophenol are applied to or may come in contact with hair, skin (particularly the scalp), eyes, and nails. Individuals may use these formulations as often as once per week.

The oxidative or permanent hair dyes containing 2-Methyl-5-Hydroxyethylaminophenol, as "coal tar" hair dye products, are exempt from the principal adulteration provision and from the color additive provision in Sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a caution statement and "patch test" instructions for determining whether the product causes skin irritation.[13] 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.

Patch test instructions call for a 24-h patch on the skin of the user with the intermediates and hydrogen peroxide mixed in the same manner as in use. This test is to be performed prior to each and every application of the hair dye.[14]

Noncosmetic Use

The aminophenols are intermediates in the manufacture of sulfur and azo dyes and are used in the dyeing of furs and feathers.[4]

TABLE 2. Product Formulation Data[12]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)		
			>1–5	>0.1–1	≤0.1
2-Methyl-5-Hydroxyethylaminophenol					
Hair dye preparations	946	54	4	28	22
1986 Totals		54	4	28	22

Percutaneous Absorption

The *in vitro* penetration of a dye base containing 0.1 mole of 2-Methyl-5-Hydroxyethylaminophenol per 0.1 mole of *p*-phenylenediamine was evaluated using abdominal skin (epidermis + dermis) of female hairless rats (Iffa Credo strain). The skin (3 cm^2 surface, epidermis facing up) was positioned between the two compartments of a simple diffusion cell. The upper compartment contained 24 mg of the dye base, 36 mg of hydrogen peroxide (dye/hydrogen peroxide ratio = 1/1.5), and 18 mg of bleached hair (cut into 5 mm lengths). This mixture remained in contact with the skin for 30 min. The lower compartment, for recovery of the 2-Methyl-5-Hydroxyethylaminophenol that penetrated the skin, contained 3 ml of 0.9% saline solution. Detection of 2-Methyl-5-Hydroxyethylaminophenol was accomplished via high-performance liquid chromatography. The average quantity (6 experiments) of 2-Methyl-5-Hydroxyethylaminophenol that penetrated the skin was 0.4 $\mu g/cm^2$ of skin surface. This quantity corresponded to 0.025% of the original quantity deposited on the skin. In similar experiments (same procedure) involving human abdominal skin (1.65 cm^2 surface, epidermis only), the epidermis was exposed to 33 mg of a mixture comprising the dye (2-Methyl-5-Hydroxyethylaminophenol + *p*-phenylenediamine) and hydrogen peroxide for 30 min. In seven experiments, the average quantity of 2-Methyl-5-Hydroxyethylaminophenol that penetrated the epidermis was 0.07 $\mu g/cm^2$ of skin surface. This quantity corresponded to 0.044% of the original quantity deposited on the skin.[15]

TOXICOLOGY

Acute Oral Toxicity

The acute oral LD_{50} for 2-Methyl-5-Hydroxyethylaminophenol was 1.35 g/kg in Swiss mice (strain: OF 1). The experimental procedure was not stated (Table 3).[16]

The acute oral toxicity of an aqueous solution of 2-Methyl-5-Hydroxyethylaminophenol (pH 3.9) was evaluated using 40 male Swiss albino mice (weights = 25–30 g). The solution was administered via oral intubation and animals were observed over a period of 14 days. The LD_{50} was 3.10 g/kg of body weight, with 95% confidence limits ranging from 2.50 to 3.84 g/kg (Table 3).[17]

The acute oral toxicity of a dye containing 2-Methyl-5-Hydroxyethylaminophenol was evaluated in 50 male and female rats (weights = 200–300 g) of the Sherman-Wistar strain. The concentration of 2-Methyl-5-Hydroxyethylaminophenol in the dye was not stated. Prior to dosing, the animals were deprived of feed, but not water, for 24 h. The dye was administered via oral gavage at doses of 1.0, 2.0, 4.0, 8.0, and 16.0 g/kg. After dosing, deaths and signs of toxicity were recorded during a 14-day period; feed and water were allowed *ad libitum*. Ten animals (5 males, 5 females) were tested at each dose. All animals given doses of 8.0 and 16.0 g/kg died; these were the only deaths reported. Lesions were not observed at necropsy. The LD_{50} was 5.7 g/kg, with 95% confidence limits ranging from 4.0 to 8.0 g/kg (Table 3).[18] Similar results were reported in an acute oral toxicity study (same methodology) or another dye containing 2-Methyl-5-Hydroxyethylaminophenol; however, the concentration of 2-Methyl-5-Hydroxyethylaminophenol in the dye was not stated.[19]

The acute oral toxicity of a hair dye containing 2-Methyl-5-Hydroxyethylaminophenol (concentration not stated), diluted in propylene glycol, was evaluated in

TABLE 3. Oral Toxicity of 2-Methyl-5-Hydroxyethylaminophenol

Type of study	*Animals tested*	*Test substance and vehicle*	*Methodology*	*Results*	*References*
Acute oral toxicity	Swiss mice, strain OF1 (no. and weights not stated)	Pure ingredient (vehicle not stated)	—	LD_{50} = 1.35 g/kg	16
Acute oral toxicity	40 Swiss albino mice (25–30 g)	Pure ingredient in aqueous solution (conc. not stated)	Oral intubation	LD_{50} = 3.1 g/kg 95% confidence limits: 2.5–3.8 g/kg	17
Acute oral toxicity	50 Sherman-Wistar rats (200–300 g)	Dye containing ingredient (conc. not stated)	Intragastric administration: doses of 1.0, 2.0, 4.0, 8.0, and 16.0 g/kg (10 rats/dose)	LD_{50} = 5.7 g/kg 19/20 confidence limits: 4.0–8.0 g/kg	18,19
Acute oral toxicity	Wistar albino rats (200/g, 10/group) and Swiss albino mice (20/g, 10/group). Total no. tested not stated.	Dye containing ingredient (conc. not stated)	Single oral dose of 3.0 g/kg via esophageal probang	No deaths	20
Subchronic oral toxicity	20 Sprague-Dawley rats (6–7 weeks old)	Dye containing ingredient (conc. not stated) in propylene glycol	Oral intubation: doses of 0.15 g/kg for 90 days	No deaths	21

Swiss albino mice (5 males, 5 females/group, 20 g) and Wistar albino rats (5 males, 5 females/group, 200 g). The total number of animals tested was not stated. Animals were deprived of feed for 16 h prior to dosing. A single dose, 3.0 g/kg, of the test solution was administered into the stomach by means of an esophageal probang. Feed was allowed immediately after administration. None of the animals died during the 7-day observation period and no lesions were noted at necropsy (Table 3).[20]

Subchronic Oral Toxicity

A suspension of a hair dye containing 2-Methyl-5-Hydroxyethylaminophenol (concentration not stated) in propylene glycol was administered to 20 Sprague-Dawley rats (male and female, 6–7 weeks old) by means of an esophageal tube. Each animal received a daily dose of 0.15 g/kg for 90 days. A group of 20 control animals received propylene glycol. There were no deaths in either experimental or control groups. A biochemical analysis of blood samples indicated no differences between control and treated animals. No treatment-related tissue alterations were observed at microscopic examination (Table 3).[21]

Ocular Irritation

The ocular irritation potential of a hair dye containing 2-Methyl-5-Hydroxyethylaminophenol (concentration not stated) was evaluated using 6 albino rabbits

(weight = 2 kg). One-tenth milliliter of the dye (2% in propylene glycol) was instilled into 1 eye of each animal; the eyes were not rinsed. The untreated eye served as the control. Examinations for signs of ocular irritation were done at 1, 2, 3, 4, and 7 days postinstillation. The average ocular irritation score at day 1 for the 6 animals was 1.66 (scale: 0–110). No signs of ocular irritation were noted at day 7. The test solution was practically nonirritating to the eyes of rabbits.[22]

Skin Irritation

The skin irritation potential of 2-Methyl-5-Hydroxyethylaminophenol (5.0% in ethanol) was evaluated using six New Zealand white rabbits. The test substance (0.5 ml) was applied to two sites on the back (abraded and intact skin) that had been clipped free of hair. Each site was covered with a gauze patch and an occlusive dressing for 24 h. Sites were then wiped and scored according to the Draize scale: 0 (no erythema) to 4 (severe erythema) and 0 (no edema) to 4 (severe edema). Sites were scored again at 72 h. The test substance was not a primary skin irritant (primary irritation index = 0.17).[23] In a similar study (same procedure) involving six New Zealand white rabbits, 2-Methyl-5-Hydroxyethylaminophenol was not a primary skin irritant when applied at a concentration of 1.6% in ethanol. The primary irritation index was 0.8 (Table 4).[23]

The skin irritation potential of a hair dye containing 2-Methyl-5-Hydroxyethylaminophenol (concentration not stated) was evaluated using 6 albino Bouscat rabbits (male and female, 2.5–2.8 kg). The dye was applied (closed patches) at a concentration of 2% in propylene glycol to the shaved left flank and shaved and abraded right flank of each animal. Patches were removed after 24 h of contact, and sites were graded 30 min later for erythema and eschar formation (combined score) and edema according to the Draize[24] scale: 0 (none) to 4 (severe). Sites were again graded 48 h after the first evaluation. The scores for abraded skin and intact skin were added together, resulting in a total of 4 scores for each of the 6 animals. This sum was then divided by 24 to determine the primary irritation index, 0.04 (max possible = 8). The hair dye was classified as a mild irritant (Table 4).[25]

In another study, the skin irritation potential of a hair dye containing 2-Methyl-5-Hydroxyethylaminophenol (concentration not stated) was evaluated using 6 albino rabbits. The test substance (0.5 g) was applied via gauze patches to abraded skin on 1 side of each animal's back, and to intact skin on the other side. Patches were removed after 24 h. Sites were graded for erythema and eschar formation (combined score) and edema 24 and 72 h after patch application according to the scale by Draize:[24] 0 (none) to 4 (severe). The hair dye was classified as a primary irritant (Table 4).[26]

Skin Irritation and Sensitization

The skin sensitization potential of 2-Methyl-5-Hydroxyethylaminophenol (5.0% in ethanol) was evaluated using 12 guinea pigs of the Hartley strain. The test substance (0.5 ml) was applied to dorsal skin that had been clipped free of hair. The application site was then covered with a gauze patch and an impervious material for 6 h. After a one-day nontreatment period, a fresh application was made to the same site. This procedure was repeated for a total of nine applications, after which a two-week nontreatment period was initiated. Skin reactions were scored after each patch removal according to the Draize scale: 0 (no erythema) to 4 (severe erythema to slight eschar

TABLE 4. Skin Irritation and Sensitization of 2-Methyl-5-Hydroxyethylaminophenol

Type of study	*Animals tested*	*Test substance and vehicle*	*Methodology*	*Results*	*References*
Skin irritation	6 New Zealand white rabbits	2-Methyl-5-Hydroxyethl-aminophenol (5% in ethanol)	24-h application (occlusive patches) to abraded and intact skin	Nonirritant	23
Skin irritation	6 New Zealand white rabbits	2-Methyl-5-Hydroxyethyl-aminophenol (1.6% in ethanol)	24-h application (occlusive patches) to abraded and intact skin	Nonirritant	23
Skin irritation	6 albino Bouscat rabbits	Dye (2% in propylene glycol) containing ingredient (conc. not stated)	24-h application (closed patches) to abraded and intact skin	Mild irritation	25
Skin irritation	6 albino rabbits	Dye containing ingredient (conc. not stated)	24-h application (0.5 g, gauze patches) to abraded and intact skin	Primary irritation	26
Skin irritation and sensitization	12 Hartley guinea pigs	2-Methyl-5-Hydroxyethyl-aminophenol (5% in ethanol)	Repeated insult patch test (gauze patches, intact skin)	Nonirritant and nonsensitizer	23
Skin irritation and sensitization	12 Hartley guinea pigs	2-Methyl-5-Hydroxyethyl-aminophenol (1.6% in ethanol)	Repeated insult patch test (gauze patches, intact skin)	Nonirritant and nonsensitizer	27
Skin sensitization	10 Hartley guinea pigs	Dye containing ingredient (conc. not stated): 50% in Freund's complete adjuvant, 25% in petrolatum	Two 48-h exposures (occlusive patches) to 50% and 25% conc., respectively, followed by one 24-h exposure to 25% conc.	Mild dispersed redness in 70% of animals; moderate potential for inducing allergenicity	28

formation) and 0 (no edema) to 4 (severe edema). During the 24-h challenge period, the test substance was applied to new sites. Reactions were scored (same scale) at 24 and 48 h postapplication. The test substance was neither a primary skin irritant nor a sensitizer.[23] In another study (same procedure) involving 12 Hartley guinea pigs, 2-Methyl-5-Hydroxyethylaminophenol was also neither a primary skin irritant nor a sensitizer when tested at a concentration of 1.6% in ethanol (Table 4).[27]

Skin Sensitization

The sensitization potential of a hair dye containing 2-Methyl-5-Hydroxyethylaminophenol (concentration not stated) was evaluated using 10 Hartley guinea pigs (5 males, 5 females, 300 g). Two applications of the test substance were made to abraded skin of the scapular region. Initially, a 50/50 emulsion of the test substance in Freund's complete adjuvant was applied via an occlusive patch (2-day contact period). After an 8-day nontreatment period, the test substance was applied at a concentration of 25% in

petrolatum. A 15-day nontreatment period was observed at the end of exposure. The test substance was then applied (one-day exposure) to the shaved hindquarter of each animal via an occlusive patch. Animals were then shaved clean, and excess test substance and petrolatum removed with ether. Test sites were scored for redness 3 h later according to the scale: 0 (no reaction) to 3 (severe redness with edema or eczematoid reaction); these scores were not reported. Sites were also scored 2 and 3 days after the first reading (same scale). Six and 7 animals had mild, dispersed redness at 2 and 3 days, respectively; reactions had cleared by day 5. The sensitization index for the hair dye was 0.6 (max = 1), interpreted as a moderate potential for inducing allergenicity (Table 4).[28]

Photoallergenicity

The photoallergenicity of 2-Methyl-5-Hydroxyethylaminophenol was evaluated using 42 male guinea pigs of the Hartley strain (weights = 316–377 g). The experimental group, as well as positive and negative control groups, consisted of ten animals each. Negative controls were treated with methanol (100 μl). Positive controls were treated with 10% musk ambrette in methanol during induction, and 0.1%, 1%, and 10% musk ambrette during challenge. Three additional groups of four guinea pigs served as challenge irritation controls for the test substance, positive control, and negative control. During induction, 0.1 ml of Freund's complete adjuvant was intradermally injected into four depilated areas on the back of the neck; the area was then stripped with cellophane. A total of five applications (100 μl each) of 5% 2-Methyl-5-Hydroxyethylaminophenol in methanol were then made to the same site on days 1, 3, 5, 8, and 10, respectively. After a 30-min nontreatment period, the sites were irradiated with a bank of eleven fluorescent black light lamps (λ = 350 nm), adjusted to deliver 10 J/cm^2 per 0.1 to 1 h of exposure. Sites were scored for erythema 24 h after each application according to the scale: 0 (no irritation) to 3 (severe erythema, with or without edema). During the challenge phase, the test substance was applied, at concentrations of 1.6% and 5% in methanol, to eight depilated sites on the lower back of each animal. The left side of the back was irradiated (same procedure) at 30 min postapplication. Sites were scored for erythema 1, 2, and 3 days after irradiation. Both positive and negative control animals were treated in a manner similar to that of experimental animals. During induction, mild erythema was observed in all groups. However, neither the test substance nor the negative control induced photoallergic responses. The positive control was a photoallergen.[29]

Phototoxicity

The phototoxicity of 2-Methyl-5-Hydroxyethylaminophenol was evaluated using 48 male hairless mice of the Skh-Hr strain (8–11 weeks old). The two experimental groups, as well as the positive and negative control groups, consisted of twelve animals each. Negative controls were treated with methanol, and positive controls with 8-methoxypsoralen. Initially, the two experimental groups were treated with 1.6% and 5% 2-Methyl-5-Hydroxyethylaminophenol in methanol, respectively. Each concentration (volume = 20 μl) was applied via a micropipette to dorsal skin. At 30 min postapplication, six animals in each group were exposed (1 h, same site) to light emanating from a long-arc, Xenon high-pressure burner (6.5 kW). The intensity of the

light was 0.5 SU/h (SU = sunburn unit) at a distance of approximately 1 m. The effective energy of 1 SU is 0.200 J/cm^2 (λ = 297 nm). Application sites of the remaining animals in both groups were irradiated for 1 h with a bank of 11 fluorescent black light lamps (λ = 350 nm, I = 0.5 SU/h at 0.27 m). Sites were scored for erythema, edema, scaling, ulceration, or fissuring at 4 h and at days 1, 2, 3, and 4 postapplication. Both positive and negative control animals were treated in a manner similar to that of the experimental groups. Neither 2-Methyl-5-Hydroxyethylaminophenol nor the negative control was phototoxic. The positive control was phototoxic to all animals in the presence of both light sources.[30]

Teratogenicity

The teratogenic potential of 2-Methyl-5-Hydroxyethylaminophenol was evaluated using 80 pregnant rats (CrL: COBS CD (SD) BR strain, 151–190 g). The test substance was suspended in 0.5% carboxymethylcellulose to prepare an 18% w/v suspension. Lower concentrations of 0.5 and 3.0% w/v were prepared by serial dilution of the highest concentration with 0.5% carboxymethylcellulose. Fresh suspensions were prepared each day and stored at room temperature prior to dosing. The 0.5%, 3%, and 18% suspensions were administered in doses of 50, 300, and 1800 mg/kg/day, respectively, via intragastric intubation on days 6 through 15 of gestation. The control group (20 animals) was given 0.5% carboxymethylcellulose. None of the dams died during the treatment period. On day 20 of gestation, the dams were asphyxiated with CO_2. Fetal visceral tissues were then prepared for microscopic examination; skeletal examinations were also performed. When compared with controls, the mean fetal weight was significantly lower ($p < 0.05$) only in the group of dams receiving 300 mg/kg/day. Embryonic and fetal development, as assessed by the incidence of minor visceral and skeletal anomalies and the distribution of skeletal variants, were unaffected by treatment.[31]

Mutagenicity

In Vitro Studies

The mutagenic potential of a hair dye containing 2-Methyl-5-Hydroxyethylaminophenol was tested using strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 of *Salmonella typhimurium* and strains WP 2, WP 2 *uvr*A$^-$, and WP 2 *uvr*A$^-$/*rec*A$^-$ of *Escherichia coli*. Tests were conducted with and without metabolic activation. The concentration of 2-Methyl-5-Hydroxyethylaminophenol in the dye was not stated. *Salmonella typhimurium* and *E. coli* strains were incubated for 2 days (37°C) and 1 day, respectively, with the following test substance concentrations: 30, 76, 189, 754 µg, and 2 mg per plate. Incubation periods were followed by examinations for precipitates and microcolony growth. Phosphate buffer and 2-aminoanthracene served as negative and positive controls, respectively. The colorant was not mutagenic to any of the bacterial strains tested (Table 5).[32] In spot tests for determining DNA damage and repair, *E. coli* strains WP2, WP2 *uvr*A$^-$, and WP2 *uvr*A$^-$/*rec*A$^-$ were incubated with the colorant for one day (37°C). Tests were conducted with and without metabolic activation. The highest concentration of the dye tested was 1 mg/plate. At the end of the incubation period, plates were examined for zones of growth inhibition. Mutagenic

TABLE 5. Mutagenicity of 2-Methyl-5-Hydroxyethylaminophenol

Strains/cells tested	*Test substance*	*Methodology*	*Results*	*References*
Salmonella typhimurium: TA1535, TA1537, TA1538, TA98, TA100 *E. coli:* WP2, WP2 *uvrA*$^-$, WP2 *urvA*$^-$/*recA*$^-$	Dye (2% solution) containing ingredient (conc. not stated). Concentrations of dye tested: 30–750 μg and 2 mg/plate	Plate test (presence and absence of metabolic activation)	Negative	32
E. coli: WP2, WP2 *uvrA*$^-$, WP2 *uvrA*$^-$/*recA*$^-$	Dye (2% solution) containing ingredient (conc. not stated). Maximum conc. of dye tested = 1 mg/plate	Spot test for DNA damage and repair (presence and absence of metabolic activation)	Negative	32
Salmonella typhimurium: TA1535, TA1537, TA1538, TA100, TA98	Ingredient in DMSO. Concentrations tested: 5–1000 μg/plate.	Ames *Salmonella*/microsome plate test (presence and absence of metabolic activation)	Negative	34
Salmonella typhimurium: TA1535, TA1537, TA1538, TA100, TA98	Ingredient in DMSO. Concentrations tested: 10–500 μg/plate.	Ames *Salmonella*/microsome plate test (presence and absence of metabolic activation)	Negative	35
Saccharomyces cerevisiae: D4	Ingredient in DMSO. Concentrations tested: 250–4000 μg/ml.	Gene conversion assay (presence and absence of metabolic activation)	Negative	35
Schizosaccharomyces pombe: P_1	Ingredient in DMSO. Concentrations tested: 10–40 mM	Gene forward mutation test (presence and absence of metabolic activation)	Negative	36
Saccharomyces cerevisiae: D4	Ingredient in DMSO. Concentrations tested: 10–40 mM.	Mitotic intragene recombination test (presence and absence of metabolic activation)	Negative	37
Chinese hamster ovary cells	Ingredient in water. Concentrations tested: 0.3, 0.6, and 1.2 mg/ml	Chromosomal aberrations assay	Negative	38
Drosophila melanogaster: wild-type (Berlin K)	Ingredient in DMSO. Concentrations tested: 25 and 100 mM	Sex-linked recessive lethal test	Negative	39
Human cancer cell line (HeLa cells)	Ingredient in DMSO. Concentrations tested: 6, 17, and 50 mM	Unscheduled DNA synthesis assay (presence and absence of metabolic activation)	Negative	40
Bone marrow erythrocytes (CD-1 strain mice)	Ingredient in distilled water	Oral doses of 1250, 2500, and 5000 mg/kg given to mice; bone marrow erythrocytes evaluated in micronucleus test	Negative	41
Bone marrow erythrocytes (Swiss mice)	Ingredient in distilled water	Intraperitoneal injections of 12.5, 25, and 50 mg/kg into mice; bone marrow erythrocytes evaluated in micronucleus test	Negative	42
Bone marrow erythrocytes (Swiss mice, OF1 strain)	Ingredient in DMSO	Intraperitoneal injections of 800, 1000, and 1200 mg/kg into mice; bone marrow erythrocytes evaluated in micronucleus test	Negative	16

effects were not noted in any of the *E. coli* strains tested. Identical results were reported when the spot tests were repeated (Table 5).[32]

The mutagenic activity of 2-Methyl-5-Hydroxyethylaminophenol was investigated in strains TA 1535, TA 1537, TA 1538, TA 100, and TA 98 of *S. typhimurium* using the Ames *Salmonella*/microsome plate test.[33] The test substance (diluted with dimethyl sulfoxide, DMSO) was evaluated at concentrations ranging from 5 to 1000 μg/plate with and without metabolic activation. Positive controls were as follows: 2-nitrofluorene, 2-aminoanthracene, and 1,2-diamino-4-nitrobenzene. The plates were incubated in the dark for 3 days (37°C), after which the number of *his*$^+$ revertant colonies per plate was determined. A dose-response relationship indicating a progressive increase over spontaneous background as a function of concentration was considered to be a reliable criterion for genotoxic activity of the test substance. The test substance did not induce mutagenic effects in any of the strains tested (Table 5).[34]

The mutagenic potential of 2-Methyl-5-Hydroxyethylaminophenol was evaluated in strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 of *Salmonella typhimurium* (*Salmonella*/mammalian microsome assay) and in strain D4 of *Saccharomyces cerevisiae* (gene conversion assay). The test substance (dissolved in DMSO) was tested in the *Salmonella* assay at concentrations ranging from 10 to 500 μg/plate according to the methods of Ames[43] and Ames et al.[33,44] Untreated cultures served as negative controls and cultures treated with 1,2-diamino-4-nitrobenzene, 2-aminoanthracene, and 2-4-diaminoanisole served as positive controls. The strains were incubated (3 plates/dose) in the presence and absence of metabolic activation for 3 days before colonies were counted. Results represented the average of two experiments. The number of revertant colonies in cultures treated with 2-Methyl-5-Hydroxyethylaminophenol was not significantly different from that of untreated cultures. The test substance was not mutagenic to any of the five strains at any of the concentrations tested. In the gene conversion assay, cellular suspensions of strain D4 were treated with test substance concentrations ranging from 250 to 4000 μg/ml with and without metabolic activation. Plates (3/dose) were incubated for 3 days before determining the number of convertant colonies. Negative control cultures were incubated with phosphate buffer and positive control cultures were incubated with ethyl methanesulfonate. The test substance was not mutagenic either with or without metabolic activation at any of the concentrations tested (Table 5).[35]

In another study, the mutagenicity of 2-Methyl-5-Hydroxyethylaminophenol (pure grade) was evaluated in a forward mutation system in the yeast, *Schizosaccharomyces pombe* (strain P_1); the test substance was dissolved in DMSO. Doses of the test substance, ranging from 10 to 40 mM, were added to yeast cell suspensions with and without metabolic activation. Untreated cultures served as negative controls. The two positive controls were 2,4-diaminoanisole and *N*-nitrosodimethylamine. After 16 h of incubation in a shaking water bath (32°C), cells were plated, incubated for 5 days (32°C), and then examined for the presence of mutants. Mutagenicity was evaluated by regression analysis of a plot of dose versus mutation frequency. The test substance did not induce mutagenic effects with and without metabolic activation (Table 5).[36]

The mitotic gene conversion (mitotic intragene-recombination) test was used to evaluate the genotoxic potential of 2-Methyl-5-Hydroxyethylaminophenol (pure grade) in the yeast, *Saccharomyces cerevisiae* (strain D4); the test substance was dissolved in DMSO. Doses of the test substance, ranging from 10 to 40 mM, were added to yeast cell suspensions with and without metabolic activation. Untreated cultures served as

negative controls. The positive controls were hycanthone and 2,4-diaminoanisole. After incubation in a shaking water bath (35°C, time not specified), cells were plated and incubated for 4 days. The survivors and gene-convertant colonies were then scored. Mutagenicity was evaluated by regression analysis of a plot of dose versus mutation frequency. The test substance was not mutagenic with and without metabolic activation (Table 5).[37]

The induction of structural chromosomal aberrations by 2-Methyl-5-Hydroxyethylaminophenol (diluted with water) was evaluated in Chinese Hamster Ovary cells. Doses of 0.3, 0.6, and 1.2 mg/ml were tested. Cells were cultured (37°C) in a medium consisting of 15% newborn calf serum and antibiotics. During the last 2 h of culture, colcemid was added to block metaphase and facilitate spreading. Cultures were then subjected to trypsinization and hypotonic shock and fixed with acetic acid-methanol (1:3). Three fixations were done at 6, 12, and 16-h intervals; cells were exposed to the test substance throughout each fixation period. At microscopic examination, chromosomal aberrations were scored in 100 cells. The test substance did not significantly raise the frequency of chromosomal aberrations above the control value. This finding was confirmed when the experiment was repeated twice (12-h fixation period) (Table 5).[38]

The mutagenic potential of 2-Methyl-5-Hydroxyethylaminophenol (pure grade) was evaluated in an unscheduled DNA synthesis assay involving a human cancer cell line (HeLa cells). The test substance was diluted with DMSO. Untreated cultures served as negative controls and cultures treated with 2,4-diaminoanisole served as positive controls. Cells were treated with 6, 17, and 50 mM concentrations of the test substance for 1 h (37°C) with and without a metabolic activation system. After washing, cells were labeled with 10 mCi/ml [^{3}H]TdR (15-25 Ci/mmol), incubated (fresh medium) for 4 h, and prepared for scintillation counting and autoradiography. Results indicated that unscheduled DNA synthesis was not induced by the test substance with and without metabolic activation (Table 5).[40]

In Vivo Studies

The mutagenic potential of 2-Methyl-5-Hydroxyethylaminophenol was evaluated in *Drosophila melanogaster* using the sex-linked recessive lethal test. The test substance (diluted with DMSO) was fed to wild-type (Berlin k) adult males via glass filters (dose = 25 mM). Control groups were fed 5% sucrose in 2% DMSO. The males were then mated individually in single culture vials (culture temperature = 25°C) with 3 virgin females (Basc genotype). After 2 to 3 days, the treated paternal flies were each transported to a new vial with fresh virgin females in order to raise the second brood. The third brood was raised according to the same procedure. After 13 to 15 days, the F_1 females were mated. The F_2 progeny was inspected for the occurence of sex-linked recessive lethals. The experiment was repeated with a test substance dose of 100 mM. At each concentration tested, data were pooled and compared with the pooled data from the controls. Comparisons were made using the Kastenbaum-Bowman significance test or the Fischer's exact test.[45,46] Results indicated no increase in the mutation frequency over that of controls (Table 5).[39]

The effect of 2-Methyl-5-Hydroxyethylaminophenol on the incidence of micronucleated polychromatic erythrocytes in bone marrow was evaluated using the micronucleus test. The test substance, prepared as a suspension in sterile distilled water, was administered via gavage to three groups of CD-1 strain mice (5 mice/group, 18–21 g) in

doses of 1.25, 2.5, and 5.0 g/kg, respectively. Each dose was administered twice at 24-h intervals. The negative control group was given sterile distilled water via gavage, and the positive control group, mitomycin C via intraperitoneal administration. Animals were sacrificed 6 h after administration of the second dose. Bone marrow smears (from femur) were then examined for the presence of micronuclei in 2000 polychromatic erythrocytes per mouse and for the ratio of normochromatic to polychromatic erythrocytes. Both the group mean micronucleated cell count and the group mean normochromatic to polychromatic erythrocyte ratios were not significantly different from the control values. 2-Methyl-5-Hydroxyethylaminophenol was not mutagenic (Table 5).[41]

The micronucleus test was used to evaluate the mutagenic potential of 2-Methyl-5-Hydroxyethylaminophenol. The test substance (in water) was administered via intraperitoneal injection to 6 pairs of male Swiss mice (10–12 weeks old) in doses of 12.5, 25, and 50 mg/kg (2 pairs/dose). Half of the animals were sacrificed 24 h after treatment, and the remaining half, 48 h after treatment. Bone marrow smears (from femur) were then examined by light microscopy to determine the incidence of micronucleated cells per 1000 polychromatic erythrocytes, 2000–6000 cells being scored at each dose level. A statistical analysis of the data was performed using the *t*-test. The test substance was not mutagenic (Table 5).[42]

Again, the mutagenicity of 2-Methyl-5-Hydroxyethylaminophenol was evaluated using the micronucleus test. The test substance was dissolved in 20% aqueous DMSO and administered intraperitoneally to male Swiss mice (OF 1 strain, 28–32 g) according to the procedures of Schmid[47] and Salomone et al.[48] In the Schmid procedure, three groups of animals (5/group) received double doses of 0.8, 1.0, and 1.2 g/kg, respectively; doses were separated by a 24-h interval. Animals were sacrificed 6 h after the second administration. In the Salomone procedure, a single dose of 1.0 g/kg was administered to four groups of 5 mice. Animals were sacrificed at 30, 48, 72, and 96 h after treatment. Mitomycin C and 20% DMSO served as positive and vehicle controls, respectively, in both experimental procedures. Also, in both procedures, bone marrow smears were performed according to the method by Schmid.[47] A total of 2000 polychromatic erythrocytes per mouse was evaluated for the occurrence of micronuclei. The standard tables of Kastenbaum and Bowman[45] were used for determining the statistical significance of differences between experimental and negative control data. For mice injected with 0.8 and 1.0 g/kg of 2-Methyl-5-Hydroxyethylaminophenol, there were no significant differences in the frequency of micronuclei in comparison with negative control groups. However, at the highest dose administered (1.2 g/kg), the test substance induced a significant increase in the frequency of micronuclei over that of the negative control group. This result was possibly due to a toxic effect resulting from the administration of a concentration that approached the LD50 (1.35 g/kg). The test substance was not mutagenic (Table 5).[16]

CLINICAL ASSESSMENT OF SAFETY

Skin Irritation and Sensitization

One-hundred adult subjects, each with a history of contact allergies, were patch-tested with a dye formulation containing 1.25% 2-Methyl-5-Hydroxyethylaminophenol (vehicle = cream base). A formulation containing 2.0% *p*-tolulenediamine

served as the positive control. Patches remained in place for 48 or 72 h. Each site was graded 30 min after patch removal according to the scale: 0 (no reaction), +? (mild erythema), 1+ (erythema and prurigo), 2+ (erythema and edema accompanied by pruritis), and 3+ (isolated or confluent vesicles, erythema, edema, pruritis). The distribution of positive reactions (5 subjects) to the test substance was as follows: 1 subject (+?), 2 subjects (1+), and 2 subjects (2+). Twelve subjects had positive reactions to the control: 1 subject (+?), 6 subjects (1+), 4 subjects (2+) and 1 subject (3+). The test substance was classified as a nonirritant, and was a less potent sensitizer than the positive control.[49]

Skin Sensitization

The sensitization potential of a haircoloring product containing 17.0% 2-Methyl-5-Hydroxyethylaminophenol (vehicle = cream base) was evaluated in 100 patients: allergic dermatitis (77 patients), nonallergic dermatitis (18 patients), various dermatoses (3 patients), and multiple sensitizations (2 patients). The vehicle had the following composition: stearic acid, sodium stearoyl sulfate, oleic diethanolamide, ethoxylated castor oil, ammonium hydroxide, and a sequestering agent. After the product was applied to the back of each subject, sites were covered with double-faced tricompartment adhesive patches (48-h contact period). Each site was evaluated 30 minutes after patch removal. Positive reactions were observed in two subjects: erythema and edema (1 subject) and erythema, edema, and vesiculation (1 subject). The authors concluded that use of the haircoloring product on perceptibly normal skin should not cause sensitization.[50]

SUMMARY

2-Methyl-5-Hydroxyethylaminophenol is a hair dye coupler used in oxidation hair dye formulations at concentrations ranging from ≤ 0.01 to 5.0%.

The *in vitro* percutaneous absorption of a dye base containing 0.1 mole of 2-Methyl-5-Hydroxyethylaminophenol per 0.1 mol of *p*-phenylenediamine was evaluated using abdominal skin of humans and hairless rats. The percent absorption of the quantity of dye deposited was 0.044% in humans and 0.025% in rats.

The acute oral LD_{50} of 2-Methyl-5-Hydroxyethylaminophenol in Swiss mice (strain: OF 1) was 1.35 g/kg. In another study, the acute oral LD_{50} (Swiss albino mice) of an aqueous solution of the ingredient was 3100 mg/kg (95% confidence limits: 2.5–3.84 g/kg). The acute oral LD50 (Sherman-Wistar rats) of a dye containing the ingredient (concentration not stated) was 5.7 g/kg (95% confidence limits: 4.0–8.0 g/kg). Toxicity was not induced in Swiss albino mice and Wistar albino rats receiving single oral doses, 3.0 g/kg, of a hair dye containing the ingredient (concentration not stated). Very slight toxicity was noted in Sprague-Dawley rats receiving daily oral doses (150 mg/kg) of a hair dye containing the ingredient (concentration not stated) for 90 days.

A hair dye (2% in propylene glycol) containing 2-Methyl-5-Hydroxyethylaminophenol did not induce ocular irritation when instilled into the eyes of albino rabbits.

Mild irritation was induced in albino Bouscat rabbits when a hair dye containing 2-Methyl-5-Hydroxyethylaminophenol was applied to abraded and intact skin for a

period of 24 h. In another study, primary irritation was observed in albino rabbits after a hair dye containing the ingredient (concentration not stated) remained in contact with abraded and intact skin for 24 h. Primary skin irritation was not observed after 2-Methyl-5-Hydroxyethylaminophenol (1.6% and 5.0% in ethanol) had been applied to the skins of New Zealand white rabbits for 24 h.

In repeated insult patch tests, 2-Methyl-5-Hydroxyethylaminophenol (1.6% and 5.0% in ethanol) did not cause skin irritation or sensitization in Hartley guinea pigs. A hair dye containing 2-Methyl-5-Hydroxyethylaminophenol (concentration not stated) had a moderate potential for inducing allergenicity when applied to abraded skin of Hartley guinea pigs at concentrations of 25 and 50%. Periods of contact ranged from 1 to 2 days.

2-Methyl-5-Hydroxyethylaminophenol (1.6% and 5.0% in methanol) did not induce phototoxicity in hairless mice of the Skh:Hr strain. Also, photoallergenic reactions were not observed in Hartley guinea pigs tested with 2-Methyl-5-Hydroxyethylaminophenol (1.6% and 5.0% in methanol).

In a teratogenicity study, 2-Methyl-5-Hydroxyethylaminophenol was administered via intragastric intubation to pregnant rats (CrL: COBS CD (SD) BR strain) at concentrations of 0.5, 3.0, and 50% (doses of 50, 300, and 1800 mg/kg/day, respectively) during days 6 through 15 of gestation. Embryonic and fetal development were unaffected by treatment.

A hair dye containing 2-Methyl-5-Hydroxyethylaminophenol (concentration not stated) was not mutagenic to strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 of *Salmonella typhimurium* and strains WP 2, WP 2 *uvr*A$^-$, and WP 2 *uvr*A$^-$/recA$^-$ of *E. coli* in plate tests (with and without metabolic activation). In the same study, the hair dye was not mutagenic to these *E. coli* strains in spot tests for determining DNA damage and repair (presence and absence of metabolic activation).

2-Methyl-5-Hydroxyethylaminophenol was not mutagenic in Ames *Salmonella*/microsome plate tests (presence and absence of metabolic activation) involving the strains of *Salmonella typhimurium* mentioned above.

In gene conversion tests (presence and absence of metabolic activation) involving strain D4 of *Saccharomyces cerevisiae,* 2-Methyl-5-Hydroxyethylaminophenol was not mutagenic. The ingredient also was not mutagenic in a forward gene mutation test involving the yeast, *Schizosaccharomyces pombe,* and in the sex-linked recessive lethal test involving *Drosophila melanogaster.*

2-Methyl-5-Hydroxyethylaminophenol did not induce unscheduled DNA synthesis in a human cancer cell line (HeLa cells).

The *in vivo* mutagenicity of 2-Methyl-5-Hydroxyethylaminophenol was evaluated using the micronucleus test. The ingredient did not induce a significant increase in the number of micronucleated cells (bone marrow smears) over that of the negative control group in Swiss mice (strain not stated) and in mice of the CD 1 strain. In another study, administration of the ingredient to Swiss mice (OF 1 strain) resulted in a significant increase in the frequency of micronuclei at the highest concentration tested. This finding may have been due to a toxic effect that resulted from the administration of a dose that approached the LD_{50}. The ingredient was not mutagenic.

In a skin irritation and sensitization study involving humans, a dye formulation containing 1.25% 2-Methyl-5-Hydroxyethylaminophenol was classified as a nonirritant and was a less potent sensitizer than the positive control. Each subject had a history of contact allergies. A haircoloring product containing 17.0% 2-Methyl-5-Hydroxyeth-

ylaminophenol had no sensitization potential in subjects with normal skin. The patients tested in this study each had either a history of dermatitis or multiple sensitizations.

DISCUSSION

2-Methyl-5-Hydroxyethylaminophenol is used in oxidative hair dyes at concentrations ranging from ≤ 0.1 to 5.0%. Studies in this report indicate that oxidative hair dyes containing this ingredient induce skin irritation in rabbits and sensitization in guinea pigs.

Oxidative hair dyes are exempt from both the color additive and principal adulteration provisions in the 1938 Food, Drug, and Cosmetic Act when cautionary statements and instructions for patch testing are conspicuously displayed on the label.

2-Methyl-5-Hydroxyethylaminophenol is neither a mutagen nor a teratogen, and only a small amount of it is absorbed through the skin. Therefore, metabolic studies were not required for determining the safety of 2-Methyl-5-Hydroxyethylaminophenol in cosmetics.

CONCLUSION

On the basis of the available animal and clinical data presented in this report, the CIR Expert Panel concludes that 2-Methyl-5-Hydroxyethylaminophenol is safe as a cosmetic ingredient in the present practices of use and concentration.

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