Safety Assessment of Basic Brown 17 as Used in Cosmetics

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All interested persons are provided 60 days from the above release date (i.e., February 15, 2021) to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to the Cosmetic Ingredient Review (CIR) will be discussed in open meetings, will be available at the CIR office for review by any interested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 Executive Director, Dr. Bart Heldreth.

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Lisa A. Peterson, Ph.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. Previous Panel member involved in this assessment: James G. Marks, Jr., M.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina L. Burnett, Senior Scientific Analyst/Writer, CIR.

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ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Basic Brown 17, which is reported to function as a hair dye in cosmetic products. The Panel reviewed the available data to determine the safety of this ingredient. The Panel concluded that Basic Brown 17 is safe in the present practices of use and concentration in hair dye products; however, the available data are insufficient to make a determination that Basic Brown 17 is safe under the intended conditions of use in other cosmetic product types.

INTRODUCTION

Basic Brown 17 is reported to function as a non-oxidative hair dye in cosmetic products, according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*).[1] This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Expert Panel for Cosmetic Ingredient Safety (Panel) typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties. It should be noted that the European Commission's Scientific Committee on Consumer Products (SCCP), now known as the Scientific Committee on Consumer Safety (SCCS), has produced several opinions from which CIR has summarized data.[2-5] Only the most recent version (which also contains data from the 2008 and 2012 opinions) and the 2004 opinion (which has data not reported in the more recent versions because the material studied was either of an unknown purity or a lower purity than the material used in the more recent studies) are cited in this report.

CHEMISTRY

Definition

Basic Brown 17 (CAS No. 68391-32-2) is the monoazo color that conforms to the structure in Figure 1.[1] It is reported to function as a direct, non-oxidative hair dye in hair coloring products.[2,3]

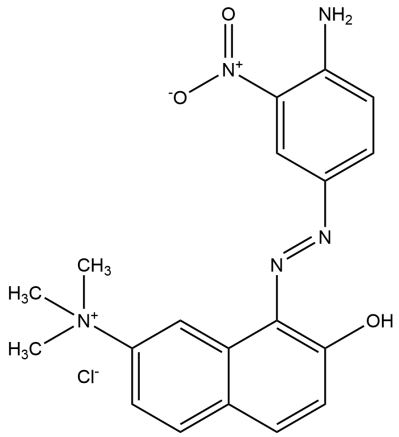

Figure 1.

Chemical Properties

Available chemical properties of Basic Brown 17 are provided in Table 1.[3,6] Basic Brown 17 is a dark brown fine powder with a formula weight of 401.85 Da (as the chloride) and an octanol/water partitioning coefficient of 2.73 at 25°C.

Method of Manufacture

No methods of manufacture were found in the public literature, and unpublished data were not provided.

Composition/Impurities

Impurities of Basic Brown 17 may include 2-nitrobenzene-1,4-diamine (also known as 2-nitro-*p*-phenylenediamine, another hair dye ingredient; < 250 ppm), Basic Red 118 (a 2-nitro isomer of Basic Brown 17; < 4.5% w/w), and 7-hydroxy-*N,N,N*-trimethylnaphthalene-2-aminium chloride (NBTRI; < 1% w/w).[3] A tradename mixture containing Basic Brown 17 may also contain saccharose to adjust color strength in formulation to a certain predefined value.

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2020 VCRP survey data, Basic Brown 17 is used in a total of 54 formulations. Of these reported uses, 3 are in non-coloring hair products (specifically a shampoo, a conditioner, and an "other" non-coloring hair product) and the remaining 51 are in coloring hair products (specifically 5 in hair dyes and colors, 22 in coloring rinses, 14 in coloring shampoos, and 10 in "other" coloring hair products).[7] The results of the concentration of use survey conducted by the Council in 2019 indicate that Basic Brown 17 is used at up to 0.66% in hair dyes and colors, up to 0.065% in coloring shampoos, and up to 0.19% in "other" hair coloring products.[8]

In the US, Basic Brown 17 is considered a coal tar hair dye for which regulations require caution statements and instructions regarding patch tests in order to be exempt from certain adulteration and color additive provisions of the US Federal Food, Drug, and Cosmetic Act. In order to be exempt, the following caution statement must be displayed on all coal tar hair dye products:

> Caution - this product contains ingredients which may cause skin irritation on certain individuals and a preliminary test according to accompanying directions should be made. This product must not be used for dyeing the eyelashes or eyebrows; to do so may cause blindness.

Product labels shall also bear patch test instructions for determining whether the product causes skin irritation. However, whether or not patch testing prior to use is appropriate is not universally agreed upon. The Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 h after application of the test material and prior to the use of a hair dye formulation. Conversely, a report in Europe suggests that self-testing has severe limitations, and may even cause morbidity in consumers.[9,10] Hair dye products marketed and sold in the US, though, must follow the labeling requirements established by the Food, Drug, and Cosmetic Act.

In the European Union, Basic Brown 17 is restricted to use only in non-oxidative hair dye products at a maximum concentration of 2.0% in ready for use preparations.[11] The European Scientific Committee on Consumer Safety (SCCS), in 2014, concluded that Basic Brown 17 is safe for use in non-oxidative hair dye formulations with a maximum concentration of 2.0%, apart from possible sensitization potential.[3] Basic Brown 17 might contain up to 4.5% (w/w) Basic Red 118; Basic Red 118 is not permitted for use in cosmetics in Europe except as an impurity in Basic Brown 17 when used as a substance in hair dye products.

TOXICOKINETIC STUDIES

Dermal Penetration

Animal

The percutaneous penetration/dermal absorption potential of Basic Brown 17 (> 94% pure) was investigated in excised pig skin that was dermatomed to 400 µm thickness.[12] The test material was studied in an aqueous solution with methanol (1:1; 10 µl/cm^2) and in a representative standard formulation (10 mg/cm^2) in which the concentration of Basic Brown 17 was 2%. The receptor solution was physiological saline and ethanol (75:25), and the exposure area of the skin disks was 2.54 cm^2. Exposure was terminated by washing of the skin surface 30 minutes after application, and the receptor fluid was analyzed at defined intervals for up to 48 h post application. The majority of the applied test material was found in the terminal rinse 30 minutes after exposure (87.7% for aqueous solution and 90.9% for standard formulation). The percutaneous penetration of Basic Brown 17 was below detection limits (0.094%) for both the aqueous solution and the standard formulation. The penetration rate was < 0.004 µg/cm^2/h. Approximately < 0.11% of the aqueous solution and < 0.16% of the standard formulation were described as bioavailable in this study.

The dermal absorption of Basic Brown 17 (77.4% pure) in a hair dye formulation at 2.0% w/w was studied in excised dermatomed pig skin.[3] The hair dye formulation (21.18 mg/cm^2, equivalent to 20 mg/cm^{2}of the test article) was applied to skin from 2 male and 2 female pigs. The skin samples were then mounted into static diffusion cells (10 replicates) containing sodium chloride (0.9% w/v) in the receptor chamber. The receptor fluid was collected at 0.5, 1, 2, 4, 6, and 24 h post-dosing. At 30 min and 24 h post-dosing, the skin surface was washed with a dilute shampoo solution and water. The skin was then removed from the static diffusion cells at 24 h, dried, and the stratum corneum was removed with 20 successive tape strips. After 24 h, the dermal bioavailability of Basic Brown 17 following topical application to pig skin was 0.48% (1.62 µg/cm^2) of the applied dose. The majority of the dose was removed by washing the skin.

Human

In a human dermal absorption study with10 male subjects, applications of 20 µl of 1 mM Basic Brown 17 in 40% aqueous isopropanol were made on 5 separate skin areas (5.3 cm^2) of the inner forearm (equivalent to about 1.5 µg/cm^2).[2] After 10 min and 24, 48, and 72 h, the test sites were subjected to 10 repeated tape strippings. During the intervals between sampling, the skin areas were protected by a special non-occlusive cover. The stripping-tapes were glued on white cardboard and kept in the dark until they were evaluated by densitometry. From the recovery rates, the amount of the test material that could possibly have penetrated the skin was estimated (details not provided). No test material was observed in the "horny layer" (stratum corneum). It was therefore concluded that Basic Brown 17 was not absorbed through the skin.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

The acute dermal toxicity of Basic Brown 17 (no vehicle; purity not reported) was studied in male and female Sprague Dawley rats.[6] Five male and 5 female rats received the test material on shaved skin at a dose of 2000 mg/kg bw. No signs of toxicity and mortality were noted during a 14-d observation period. The animals exhibited normal body weight gain through the study period of 14 d, and no abnormalities related to treatment were observed during gross pathological examination. The acute dermal LD_{50} of Basic Brown 17 was greater than 2000 mg/kg bw.

Oral

In an acute oral toxicity study, CF1 mice received Basic Brown 17 (purity not reported) once by gavage at three dose levels up to 5000 mg/kg bw, at a volume of 0.2 ml/10 g bw.[2] All animals were observed for a period of 7 d. During the observation period, no mortalities were recorded. The LD_{50} was reported to be greater than 5000 mg/kg bw in mice.

The acute oral toxicity of Basic Brown 17 (purity not reported) was studied in female Sprague Dawley rats.[6] Groups of 6 animals received Basic Brown 17 in distilled water at doses of 300 or 2000 mg/kg bw. No signs of toxicity or mortality were observed in any of the treated animals. Gross pathological examination did not reveal any abnormalities in any of the test animals. The acute oral LD_{50} of Basic Brown 17 was greater than 2000 mg/kg bw in rats.

In another acute oral study, the toxicity of Basic Brown 17 (purity not reported) was studied in groups of 4 male and 4 female CFY rats.[3] The rats received 0, 100, 1000, 4000, 8000, or 16,000 mg/kg bw Basic Brown 17 in 1% aqueous methylcellulose in a volume of 1 – 40 ml/kg bw. Clinical signs of toxicity observed during the 14 d after dosing were lethargy, piloerection, decreased respiratory rate, and hunched posture. Two male rats and 1 female rat in the 16,000 mg/kg bw dose group died. The LD_{50} in this study was determined to be between 8000 and 16,000 mg/kg bw.

Subchronic Toxicity Studies

Oral

In a 90-d feeding study, groups of 10 female CF1 mice received 1250, 2500, or 5000 mg/kg of Basic Brown 17 (purity not reported) mixed with diet.[2] A control group of 20 animals received untreated feed. All mice, with the exception of one animal in the highest group (5000 mg/kg), survived the treatment period. No changes in behavior were noted in the test group animals when compared to the controls. Feed intake and the results of hematological and biochemical tests were also comparable to controls. A decrease in body weight gains was observed in all treated groups, but based on a graphical presentation in data submitted to the SCCP, the reductions in body weight were not considered to be dose-related. No differences were observed in absolute or relative organ weights between control and treated animals. Yellow-brown urine was noted in all treated animals, which indicated gastrointestinal absorption of the test material. A yellow-brown discoloration of the stomach and intestines were observed macroscopically, and fatty infiltration of the liver and slight hemosiderosis in the spleen was noted in all the treated animals. It was concluded that dietary administration of 1250 mg/kg/d Basic Brown 17 was borderline for possible toxic effects in mice.

The potential adverse effects of Basic Brown 17 (77.4% pure) was investigated in a 90-d oral toxicity study in Wistar Hannover rats.[3] The study was performed in accordance with Organization for Economic Co-operation and Development (OECD) test guideline (TG) 408. Groups of 10 male and 10 female rats received 0, 60, 120, or 180 mg/kg/d of the test material in distilled water via gavage at 10 ml/kg bw. An extra 5 animals per sex were used for the control and high dose groups to assess recovery for 4 wk after the treatment period concluded. Two high dose males and 1 high dose female died during the treatment period. A necropsy of these animals found incomplete lung collapse in the female, with both lungs and the thymus dark/red in color. The necropsy of the males found the lungs, thymus, spleen, and thyroid dark/red in color, and one had irregularities of the heart, liver, and prostate. No treatment-related changes in body weight gains and feed consumption were observed at up to 180 mg/kg/d. No clinical signs of toxicity were observed in any of the treated animals. No significant hematological changes were noted during the study. Moderate to marked alteration in aspartate aminotransferase was reported in 3 females and 3 males in the high dose group and in 2 females in the mid-dose group. Significantly raised gamma-glutamyl transferase, cholesterol, triglycerides, glucose levels, and lowered sodium were observed in the high dose group, but these parameters were similar to the controls at the end of the recovery period. Some of

the high dose animals exhibited bilirubinuria, which was attributed to either the test substance or its metabolites found in the urine.

Necropsy of the treated animals found dark coloration in the brain, heart, kidneys, ovaries, skeletal muscle, spleen, and thyroid in the high dose rats, with the females more affected than the males. In the mid-dose group, both sexes had dark coloration of the spleen and thyroid, with some females exhibiting dark coloration in the heart and skeletal muscle. These effects were present after 4 wk of recovery. Yellow/brown pigmentation was observed in the heart, kidneys, liver, spleen, thyroid, Peyer's patches, and skeletal muscle of animals of both sexes that received $\geq$ 120 mg/kg bw/d when compared to controls. Yellow/brown pigmented macrophages were also observed in the lungs of females receiving $\geq$ 120 mg/kg bw/d. Males and females in the 180 mg/kg bw/d dose group and the recovery high dose group had yellow/brown pigmentation in the adrenals, ovaries, uterus, mesenteric/cervical lymph nodes and thymus. An increased incidence of extramedullary hemopoiesis in the spleen in all treated groups was observed. Absolute and relative thyroid weights were lower than the controls in females in the high dose group at the end of the recovery period; this group also had relative liver weights that were higher than controls. The no-observed-adverse-effect-level (NOAEL) for Basic Brown 17 (77.4% pure) in this study was calculated to be 46 mg/kg/d.[3]

In a 15-wk study, Basic Brown 17 (68% as chloride; dissolved in water) was administered 5 d/wk, by gavage, to 3 groups of 10 male and 10 female Sprague Dawley rats at doses of 50, 150, and 450 mg/kg bw.[2] Another group of 10 male and 10 female rats was given vehicle alone, and served as the control group. No adverse effects or mortalities occurred at doses of 50 or 150 mg/kg bw. Mortalities occurred at 450 mg/kg bw, either following general or central nervous system signs of toxicity, or without previous abnormal observations. Histological examination of the liver revealed individual pigment inclusions within Kupffer cells of some female rats given 50 mg/kg bw. At 150 mg/kg bw, deposits were seen in a number of tissues, but there were no accompanying degenerative or inflammatory changes. Examination of recovery groups (details not provided), maintained for a further 7 wk without treatment, showed that the deposits were persistent at 150 and 450 mg/kg bw/d, but not at 50 mg/kg bw/d. The NOAEL of this study was determined to be 150 mg/kg bw/d, and the no-observed-effect-level (NOEL) was determined to be 50 mg/kg bw/d.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

Oral

In an oral teratogenicity study of Basic Brown 17 (77.4% pure), groups of 25 female Wistar HsdBrlHan rats received the test material in distilled water via gavage at doses of 0, 60, 120, or 240 mg/kg bw/d on day 5 through day 19 of gestation.[3] The animals were checked daily for clinical signs of toxicity, abortions, premature deliveries, and mortalities. Body weights and feed consumption were determined at periodic intervals throughout the study. All animals surviving to day 20 of gestation were necropsied, and fetuses were removed and studied.

One female died in the high dose group on gestation day 11. A macroscopic examination found enlarged adrenals, abnormal swollen intestinal tract content, and a dark color of the liver and spleen. In the remaining dams, scabs and hair loss were observed in the treated females, and occasionally in the controls. Abrasion and aggressive behavior were noted in 2 high dose females on gestation days 19 and 20, respectively. Dyspnea was observed in 1 low dose female on gestation day 7. No other adverse reactions to treatment were noted in the daily observations. Statistically significant reductions in body weights gains and feed consumption were noted in the high dose group on days 9 and 12, when compared to controls. Statistically significantly lower terminal body weight and absolute weight gain were observed in the high dose group when compared with the controls. Gravid uterus weights were not affected by the treatment. At necropsy, the spleen was dark and occasionally swollen in the high dose group, which was likely due to the color of the test material. Mean values for corpora lutea, implantations, litter sizes, live fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses were similar to the controls. There were no dead fetuses. Small fetuses (total of 13) were observed in the control (4), low dose (3), mid-dose (5), and high dose (1) groups. One mid-dose fetus had enlarged brain ventricles; this effect was considered incidental. No other adverse effects considered to be treatment-related were observed in the fetuses. For the test material, the maternal NOAEL was considered to be 120 mg/kg bw/d and the fetal NOAEL was > 240 mg/kg bw/d; when taking into account the purity of the test material, the NOAELs were 93 mg/kg bw/d and > 186 mg/kg bw/d, respectively.[3]

In another oral teratogenicity study, a group of 24 pregnant Sprague-Dawley CD rats received 50 mg/kg Basic Brown 17 (68% as chloride) via gavage daily on days 6 to 15 of gestation.[2] A control group of 26 rats received the vehicle alone (distilled water). On gestation day 20, the rats were killed and Caesarean sections were performed. The number of implantation sites, resorptions, living fetuses, and the number of corpora lutea were counted in each litter. The weights of the placenta, uterus, fetuses, and dams, and the sex of the fetuses, were recorded. About one third of each litter was prepared and examined for soft tissue anomalies. The remaining fetuses were examined for skeletal abnormalities. The body weight gains were determined for each dam. No mortalities were reported in the dams. No differences in mean body weight gain were seen during the course of gestation in any group. There were no treatment-related effects concerning reproduction data or malformations of the fetuses. The level of skeletal variation or ossification in the test and control group was comparable. Basic Brown 17 was not considered teratogenic in rats at a dose of 50 mg/kg bw/d.

GENOTOXICITY STUDIES

In vitro and in vivo genotoxicity studies on Basic Brown 17 summarized here are detailed in Table 2. Genotoxicity was observed in Ames tests, a micronucleus test in human hepatoma (HepG2) cells, and in a comet assay in HepG2 cells.[2,3,6,13] Test results were negative for genotoxicity in mouse lymphoma assays (*tk* and *hprt* loci), a micronucleus test in Chinese hamster V79 cells, and a comet assay in reconstructed human skin tissue.[3] Basic Brown 17 was not clastogenic and/or aneugenic in a mouse erythrocyte micronucleus assay when tested at 5000 mg/kg bw via gavage.[2]

CARCINOGENICITY STUDIES

No carcinogenicity studies were found in the published literature, and unpublished data were not submitted.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

The dermal irritation potential of Basic Brown 17 (no vehicle; purity not reported) was assessed using 5 male and 5 female Sprague Dawley rats.[6] The test material was applied at 2000 mg/kg bw to shaved skin under an occlusive patch for 24 h. No signs of skin reactions were noted in a 14-d observation period. The test material produced a primary irritation index of 0.0 and was classified as non-irritating.

In a primary skin irritation/corrosion study, 3 male New Zealand White rabbits received 0.5 g of Basic Brown 17 (96.3% pure) moistened in water on shaved skin for 4 h under semi-occlusive patches.[3,6] The study was carried out in accordance with OECD TG 404. Observations were made 1, 24, 48, and 72 h after exposure. Very slight erythema and/or very slight edema and/or slight edema were observed on the treated areas, which resolved within 48 h. Yellow-brown staining of the treated skin by the test material was noted throughout the observation period. The study authors considered Basic Brown 17 to be not irritating in this study.

In a dermal irritation study performed in accordance with OECD TG 404, 100% Basic Brown 17 was applied undiluted (0.5 g/in^2) to shaved intact or scarified skin on the back of 6 albino rabbits of each sex.[2] The test site was covered by a linen cloth and plastic foil and left in place for 24 h. Readings were made upon removal of the test material, and then daily for the following 14 d. No irritation was observed.

Sensitization

Animal

The sensitization potential of Basic Brown 17 (purity = 77.4% by nuclear magnetic resonance spectroscopy (NMR)) was assessed in a local lymph node assay (LLNA) in 5 groups of 4 female mice.[3,6] The mice received the test material daily at concentrations of 0.2%, 0.5%, 1%, 3%, or 6% (w/v) in ethanol/water (7/3; v/v) by topical application to the dorsum of each ear lobe for 3 consecutive days. Two negative control groups, each of 4 female mice, were treated with the vehicle (ethanol/water (7/3; v/v)) only. Three positive control groups of 4 mice each were treated with 5%, 10%, and 25% (w/v) α-hexylcinnamaldehyde in acetone:olive oil (4:1, v/v) in a separate study. All treated animals survived the treatment period. No clinical signs of toxicity were observed in any animals of the control groups, the 0.2%, the 0.5%, or the 1% dose groups. On the third application day, slight erythema was observed at both dosing sites in all the mice of the 3% dose group. In the 6% dose group starting the second application day, moderate or slight erythema was observed at both dosing sites in all mice, persisting for the remainder of the in-life phase of the study. The stimulation indices (SI) for the 0.2%, 0.5%, 1%, 3%, and 6% dose groups were 1.0, 1.0, 1.3, 0.9, and 1.3, respectively. In the positive controls, the SI for 5%, 10%, and 25% were 2.4, 3.6, and 11.2, respectively. Effects noted in the 3% and 6% dose groups were determined to be due to irritation and not allergenic sensitization. The authors of the study concluded that Basic Brown 17 was not a sensitizer.[3,6]

In another LLNA performed in manner similar to that described above, 3 groups of 4 female mice received 1%, 5%, or 25% Basic Brown 17 (purity > 94%) topically.[2] The SI were 0.6, 0.7, and 1, respectively. A control group of 4 mice received water. It was concluded that Basic Brown 17 was not a sensitizer.

In a guinea pig maximization study, 10 female Dunkin-Hartley guinea pigs received Basic Brown 17 (purity not reported) in water at 0.1% w/v and as a 1:1 mixture with a solution Freund's complete adjuvant in water during intradermal induction (0.1 ml), 75% w/v during topical induction (0.4 ml; occluded for 48 h), and 25% w/v in distilled water during topical challenge (0.1 ml).[2] Reactions consisting of erythema with slight edema were observed on the skin of 7 animals. To further evaluate the reactions, a second topical application was made 1 wk later using 0.1 ml of Basic Brown 17 at a concentration of 5% in distilled water. Erythema was observed on the skin of 2 animals at 24 h (only), and at 48 h (only) in a third animal. The authors of the study did not consider the test material sensitizing despite the observed reactions. In 2004, the SCCP determined that this study is inadequate due to the intradermal induction concentration being too low.

OCULAR IRRITATION STUDIES

In Vitro

The ocular irritation potential of Basic Brown 17 (purity not reported) was determined by the MatTek EpiOcular™ model in accordance with OECD TG 492.[6] Tissues were exposed to the test material (neat) and positive and negative controls (sterile ultrapure water and methyl acetate, respectively) for 30 min. The exposure was followed by a 12-min post-soak and approximately 2 h recovery after the post-soak. The viability of each tissue was determined by a 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Basic Brown 17 was predicted to be non-irritating to eyes.

Animal

The ocular irritation/corrosion potential of Basic Brown 17 (no vehicle; 96.3% pure by high-performance liquid chromatography (HPLC)) was assessed using 3 male New Zealand White rabbits in accordance with OECD TG 405.[3,6] A single instillation of the test material (45 mg or approximately 0.1 ml) to unrinsed eyes resulted in effects on the iris in 2 animals and on the conjunctivae in all 3 animals. Iridial irritation grade 1 was observed, and resolved within 24 or 72 h. Irritation of the conjunctivae consisted of redness, chemosis, and discharge, which resolved within 7 d in all animals. Remnants of the test material were present on the outside of the eyelids 24 and 48 h after instillation in 1 animal. Yellow-brown staining on the fur caused by the test substance was noted. The study authors considered Basic Brown 17 to be non-irritating in this study.

In another ocular irritation study in rabbits, 0.1 ml of a 0.5 % (w/v) Basic Brown 17 in saline solution was instilled into the conjunctival sac of the left eye of each of 3 male and 3 female New Zealand White rabbits.[2] The right eye was treated with 0.1 ml of the vehicle and served as a control. The test material was not rinsed out. Reactions were read 30 and 60 min and 1 and 2 d following instillation of the test material, and were evaluated by the Draize method. Discoloration of the conjunctivae by the test substance was noted. No effects were observed to the cornea or the iris of any of the animals.

CLINICAL STUDIES

Case Report

A 57-year-old woman presented with eczema of the hands and feet.[14] The patient was a former hairdresser that still occasionally performed hair care services. The patient reported that she had a history of severe itching on the hands and in the ears, accompanied with a "bad taste" in the mouth, following use of a brand-name hair dye containing Basic Brown 17. Previous patch tests were positive for *p*-phenylenediamine, nickel, chromium, cobalt, and colophonium. The patient was patch tested again and had positive reactions to *p*-toluenediamine, methyldibromo glutaronitrile, and several extracts of a "hypoallergenic leather." Skin prick testing was performed with the brand-name hair dye and its ingredients. Strong positive reactions were observed within 15 min to the hair dye and to Basic Brown 17 (1% aq.; ++ reaction). Repeated testing 2 mo later with just Basic Brown 17 resulted in another ++ reaction.

MARGIN OF SAFETY

The SCCS calculated the margin of safety for a hair dye (non-oxidative) containing 2% Basic Brown 17 (on-head concentration) to be 1000.[3] This calculation is based on an adjusted NOAEL of 23 mg/kg bw/d (46 mg/kg bw/d with a bioavailability of 50%) and a systemic exposure dose (SED) of 0.023 mg/kg bw (skin area surface of 580 cm^2 x absorption through skin of 2.37 $\mu g/cm^2$ x 0.001/typical human bw of 60 kg).

HAIR DYE EPIDEMIOLOGY

Hair dyes may be broadly grouped into oxidative (permanent) and direct (semi-permanent) hair dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes are a preformed color. Basic Brown 17 is a direct, non-oxidative hair dye ingredient. While the safety of individual hair dye ingredients is not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer. A detailed summary of the available hair dye epidemiology data is available at https://www.cir-safety.org/cir-findings.

SUMMARY

Basic Brown 17 is reported to function as a hair dye in cosmetic products. According to 2020 VCRP survey data, Basic Brown 17 is used in a total of 54 formulations. Of these reported uses, 3 are in non-coloring hair products (specifically a shampoo, a conditioner, and an "other" non-coloring hair product), and the remaining 48 are in coloring hair products (specifically 5 in hair dyes and colors, 22 in coloring rinses, 14 in coloring shampoos, and 10 in "other" coloring hair products). The results of the concentration of use survey conducted by the Council in 2019 indicate that Basic Brown 17 is used at maximum concentrations of up to 0.66% in hair dyes and colors, up to 0.065% in coloring shampoos, and up to 0.19% in "other" hair coloring products.

In dermal penetration studies of Basic Brown 17 (2%) in excised dermatomed pig skin, 0.11% of the aqueous test material and 0.066% of the representative formulation was absorbed in one study, and 0.48% of the formulation was absorbed in another study. In a human dermal absorption study, Basic Brown 17 was not absorbed through the skin when 1 mM of the material in 40% aqueous isopropanol was tested.

The acute dermal LD_{50} of Basic Brown 17 in rats was greater than 2000 mg/kg bw. The acute oral LD_{50} of Basic Brown 17 in mice was greater than 5000 mg/kg bw and in rats was between 8000 and 16,000 mg/kg bw.

In a 90-d feeding study in mice that received 1250, 2500, or 5000 mg/kg Basic Brown 17, a decrease in body weight gains and fatty infiltration of the liver and slight hemosiderosis of the spleen was observed in all treatment groups. The decrease in body weight gains was not dose-related. The NOAEL for Basic Brown 17 (77.4% pure) was 46 mg/kg/d in a 90-d oral toxicity study in rats. Adverse effects included an increased incidence of extramedullary hemopoiesis in the spleen in all treated groups. Absolute and relative thyroid weights were lower than the controls in females in the high dose group at the end of the recovery period; this group also had relative liver weights that were higher than controls. In a 15-wk study in rats, the NOAEL of Basic Brown 17 (68% as chloride) was determined to be 150 mg/kg bw/d and the NOEL was determined to be 50 mg/kg bw/d (the lowest dose tested).

In an oral teratogenicity study of Basic Brown 17 (77.4%), the maternal NOAEL was 120 mg/kg/d and the fetal NOAEL was > 240 mg/kg/d, which was corrected to 93 mg/kg/d and > 186 mg/kg bw/d when accounting for the purity of the test material. Maternal effects included a statistically significant lower terminal body weight and absolute weight gain and swollen spleens in the high dose group. In another oral teratogenicity study in rats, Basic Brown 17 (68% as chloride) did not produce adverse developmental effects when tested at 50 mg/kg bw/d.

Genotoxicity was observed in Ames tests, a micronucleus test in HepG2 cells, and in a comet assay in HepG2 cells. Test results were negative for genotoxicity in mouse lymphoma assays (*tk* and *hprt* loci), a micronucleus test in Chinese hamster V79 cells, and a comet assay in reconstructed human skin tissue. Basic Brown 17 was not clastogenic and/or aneugenic in a mouse erythrocyte micronucleus test when tested at 5000 mg/kg bw by gavage.

In dermal irritation studies in rats and rabbits, Basic Brown 17 (96.3% pure) was not irritating. Basic Brown 17 (purity > 94%) at up to 25% was not sensitizing in LLNA studies in mice. Basic Brown 17 was predicted to be non-irritating to human eyes in an EpiOcular™ study, and it was not irritating in rabbit eyes when the test material was tested neat or at 0.5% in saline solution.

A case study was reported in a former hairdresser that had eczema of the hands and feet following exposure to a hair dye containing Basic Brown 17. Skin prick test were positive for the hair dye and Basic Brown 17 (1% aq.).

The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer.

No method of manufacturing or carcinogenicity studies were found in the published literature, and unpublished data were not submitted.

DISCUSSION

Basic Brown 17 is reported to function as a direct, non-oxidative hair dye in hair coloring products. The Panel recognizes that hair dyes containing this ingredient, as coal tar hair dye products, are exempt from certain adulteration and color additive provisions of the Federal 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 Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures. The Panel considered concerns that such self-testing might induce sensitization, but agreed that there was not a sufficient basis for changing this advice to consumers at this time.

In considering hair dye epidemiology data, the Panel concluded that the available epidemiology studies are insufficient to scientifically support a causal relationship between hair dye use and cancer or other toxicological endpoints, based on lack of strength of the associations and inconsistency of findings. Use of direct hair dyes, while not the focus in all investigations, appears to have little evidence of any association with adverse events as reported in epidemiology studies.

The Panel expressed concern over the mixed results in the genotoxicity studies and the lack of carcinogenicity studies. However, the Panel noted that the toxicokinetic studies show that Basic Brown 17 does not absorb through the skin and that a conservative margin of safety calculation yielded a result of 1000. These findings, coupled with the short exposure time as a rinse-off product, helped mitigate the Panel's concern.

The Panel has determined that the data are sufficient to support safety of this ingredient in hair dye products, which are rinsed-off after application. However, the Panel noted use has been reported for Basic Brown 17 in non-coloring hair products, and the data are not sufficient to make a determination of safety for uses in other cosmetic product types, especially those that may potentially lead to longer exposure duration on the skin. The additional data need to determine safety of this cosmetic ingredient in non-hair dye products are:

- Concentration of use and reported function in the non-hair coloring product uses that were reported to the FDA VCRP database
- Dermal irritation and sensitization data at maximum use concentration for non-hair coloring products

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Basic Brown 17 is safe in the present practices of use and concentration in hair dye products. However, the available data are insufficient to make a determination that Basic Brown 17 is safe under the intended conditions of use in other cosmetic product types.

TABLES

Table 1. Chemical properties for Basic Brown 17

Property	Value	Reference
Physical Form	Dark brown fine powder	[3]
Formula Weight (g/mol; as chloride salt)	401.85	[3]
Vapor Pressure (mmHg at 25°C)	0	[6]
Melting Point (°C)	200 - 202	[3]
Boiling Point (°C at 729.9 mmHg)	> 240	[6]
Water Solubility (g/l at 20°C and pH = 5.6)	16.1	[3]
log $P_{o/w}$ (temperature not given)	-0.1466	[3]
(at 25°C)	2.73	[6]
λ_{max} (nm)	216, 462	[3]

Table 2. Genotoxicity studies

Concentration/Dose/Vehicle	Species/Strain/Cell	Method	Results	Reference
In Vitro				
3-5000 µg/plate in deionized water or dimethyl sulfoxide (DMSO); purity = 77.4% (NMR)	*Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535, and TA 1537, with and without metabolic activation	Ames test; positive and negative controls were in accordance with OECD TG 471	Mutagenic; a substantial and dose-dependent increase in revertant colony numbers was observed following treatment in strains TA 98 and TA1537, with and without metabolic activation	[3,6]
4-5000 µg/plate in DMSO; purity = 68% as chloride	*S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538; with and without metabolic activation	Ames test, in accordance with OECD TG 471; appropriate positive and negative controls were used	Mutagenic; dose-related relevant increase in revertant numbers observed in strains TA 98, TA 100, TA 1537, and TA 1538, with and without metabolic activation	[2]
Experiment I: 8.1-97.5 µg/ml without metabolic activation and 16.3-198.0 µg/ml with metabolic activation Experiment II: 8.0-192.0 µg/ml without metabolic activation Tested in deionized water; purity = 77.4% (NMR)	L5178Y mouse lymphoma cells	Mouse lymphoma assay (*tk* locus), in accordance with OECD TG 476; appropriate positive and negative controls were used	Not mutagenic; no biologically-relevant and concentration dependent increase in the number of mutant colonies was observed with either experiment, with or without metabolic activation	[3]
Experiment I: 3.1-99.2 µg/ml without metabolic activation and 6.2-198.4 µg/ml with metabolic activation Experiment II: 3.1-49.6 µg/ml without metabolic activation and 3.1-99.2 µg/ml with metabolic activation Tested in deionized water; purity = 98.7% (area by HPLC)	L5178Y mouse lymphoma cells	Mouse lymphoma assay (*hprt* locus), in accordance with OECD TG 476; appropriate positive and negative controls were used	Not mutagenic; no biologically-relevant increase of mutant frequency was observed in either experiment, with or without metabolic activation	[3]
Experiment I: 31.3-4100.0 µg/ml without metabolic activation and 128.1-2050.0 µg/ml with metabolic activation Experiment II: 100.0-512.2 µg/ml without metabolic activation and 128.1-1025.0 µg/ml with metabolic activation Tested in deionized water; purity = 77.4% (NMR)	Chinese hamster V79 cells	Micronucleus test in accordance with OECD TG 487 (draft); appropriate positive and negative controls were used	Not genotoxic; test substance did not induce an increase in micronucleated cells	[3]
Experiment I: 20-500 µg/ml Experiment II: 100-2000 µg/ml; with and without metabolic activation Tested in DMSO; purity = 68% as chloride	Chinese hamster V79 cells	Mammalian cell gene mutation test (HPRT locus); appropriate positive and negative controls were used	Test material induced some increased mutant frequencies; however, precipitates were observed with metabolic activation lead to an error in the assessment of doses; the assay was considered unsuitable for genotoxicity evaluation by the SCCP	[2]

Table 2. Genotoxicity studies

Concentration/Dose/Vehicle	Species/Strain/Cell	Method	Results	Reference
Experiment I: 25-2500 µg/ml Experiment II: 3.33-333.33 µg/ml Experiment III: 0.03-3.33 µg/ml; incubated 3 h with ^{3}H-thymidine Tested in 0.9% NaCl, 68% of test material as chloride	Wistar rat hepatocytes	Unscheduled DNA synthesis (UDS) test in accordance with OECD TG 482; DMSO was negative control and 2-acetylaminofluorene was positive control	The authors concluded that Basic Brown 17 did not induce significant increases in DNA repair; however, the study was considered unsuitable for genotoxicity evaluation by the SCCP due to improper methodology	[2]
3.9, 7.8, or 15.6 µg/ml, dissolved in sterilized bi-distilled water and minimal essential medium	HepG2 cells isolated from human hepatoma	Cytokinesis-block micronucleus test; positive and negative controls utilized (no details)	Genotoxic; significant chromosomal damage was induced	[13]
3.9, 7.8, or 15.6 µg/ml, dissolved in sterilized bi-distilled water and minimal essential medium	HepG2 cells isolated from human hepatoma	Comet assay; positive control was methyl methanesulfonate in minimal essential medium (MEM) and the negative control was MEM with 1% of sterile water	Genotoxic	[13]
2000-8000 µg/ml in 70% ethanol; purity = 98.7% (area by HPLC; 77.4% by NMR)	Phenion® full-thickness reconstructed human skin tissue	Single-cell gel/Comet assay; appropriate positive and negative controls were included (no details)	Not genotoxic	[3]
In Vivo				
0 and 5000 mg/kg bw in 0.9% NaCl; test material purity was 68% as chloride	Groups of 5 male and 5 female CFW 1 mice	Mammalian erythrocyte micronucleus test in accordance with OECD TG 474; single dose via gavage; appropriate negative and positive controls were used	Not clastogenic and/or aneugenic; no clinical signs of toxicity were observed; a slight change in PCE/NCE ratio was observed, but no statistically significant increase in the frequency of PCE; controls yielded expected results	[2]

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