Safety Assessment of Basic Red 76 as Used in Cosmetics

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date to comment on this safety assessment and to identify additional published data that should be included or provide unpublished data which can be made public and included.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 All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a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

The 2018 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Priya Cherian, Scientific Analyst/Writer.

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INTRODUCTION

This scientific literature review is the initial step in preparing a safety assessment of the ingredient, Basic Red 76, in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook (*wINCI; *Dictionary*), Basic Red 76 (CAS No. 68391-30-0) is a monoazo color that functions as a hair colorant and hair-conditioning agent.[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This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated.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 CIR typically evaluates, is provided on the CIR website (http://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; http://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Much of the data included in this safety assessment was gathered from the opinions of European scientific committees, Scientific Committee on Consumer Safety (SCCS)[2] and Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP).[3]

CHEMISTRY

Definition and Structure

As given in the *Dictionary*, Basic Red 76 is the azo dye that conforms to the following structure[1]:

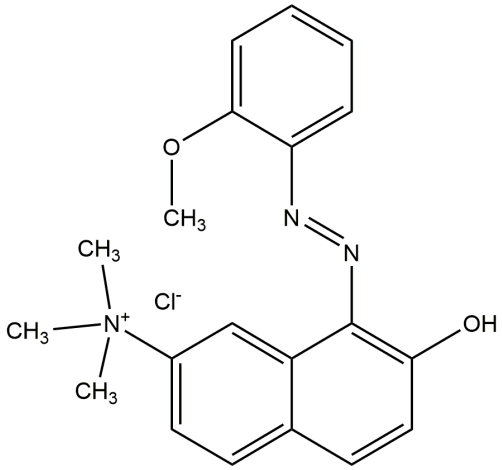

Figure 1. The azo dye, Basic Red 76

Physical and Chemical Properties

Basic Red 76 (CAS No. 68391-30-0) is a cationic direct dye that is water soluble. Ultraviolet-visible (UV-Vis)-spectra showed maxima at the wavelengths 235 nm (62% Basic Red 76) and 332 nm (80% Basic Red 76).[2] A list of chemical and physical properties for Basic Red 76 can be reviewed in Table 1.

Method of Manufacture

While no methods were found in the publically available literature specific to the preparation of Basic Red 76, most azo dyes are synthesized in the same manner.[4] The first of two steps in the classic synthesis of dyes like Basic Red 76 involves the diazotization of a primary aromatic amine (e.g., 2-methoxyaniline), in a cold aqueous, acidic solution, with sodium nitrite. The resulting diazonium salt is highly reactive, and an arylazo-dehydrogenation reaction with an aromatic alcohol (e.g., 7-hydroxy-*N*,*N*,*N*-trimethylnaphthalen-2-aminium chloride) quickly results in an azo dye.

Impurities/Components

Based on data obtained from the SCCS, it appears that the materials tested were not always purely Basic Red 76, and had various chemical compositions.[2] In some of these test material formulations, sugars were included. Monomethyl sulfate was often a component in the test material that was used as an anion to the dye. Some other reported impurities/components of the test materials include *o*-anisidine, chloride, and sodium.

Table 2 provides information on the components of the specific test materials used in the toxicity studies presented in this report.

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. Use concentration 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 the 2018 VCRP survey data, Basic Red 76 is reported to be used in 46 hair-coloring formulations.[5] The results of the concentration of use survey conducted by the Council indicate that the highest concentration of use reported for Basic Red 76 was 0.35% in hair dyes and colors.[6] Data regarding concentration and frequency of use can be reviewed in Table 3. Because this ingredient is purely a colorant, the concentration and frequency of use table provides information specific to the colorant uses.

Basic Red 76 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 U.S. 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.[7] 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.[8]

Basic Red 76 is listed in the EU Cosmetics Regulation 1197/2013 Annex III, and is allowed in non-oxidative hair dye products at a maximum concentration of 2%.[9] According to the SCCS, Basic Red 76 containing up to18% methyl sulfate does not pose a risk to the health of the consumer when used as a non-oxidative hair dye with a maximum head-on concentration of 2.0%.[2]

TOXICOKINETIC STUDIES

Azo bond cleavage and reduction is mediated by enzymes found in the liver, skin, and intestines.[10] Responsible enzymes include nicotinamide adenine dinucleotide (NADH), cytochrome P450 reductase, and NAD(P)H quinone oxidoreductase. Both skin and intestinal microflora have been shown to reduce azo linkage, forming aromatic amines (e.g., *o*-anisidine). The produced aromatic amines can potentially have a greater expected absorption rate than the dye from which they are derived from.

Dermal Penetration

Animal

A two-part dermal/percutaneous absorption study was performed according to Organization for Economic Cooperation and Development (OECD) test guideline (TG) 428.[2,11] Four replicates from each animal (one male, one female) of dermatomed pig skin, 0.75 mm thick, were used per experiment. In experiment A, 2% test material (80.5% Basic Red 76; see Table 2 for the remainder of the test material composition) in direct dye was applied to skin samples. Experiment B involved 2% test

material (80.5% Basic Red 76; see Table 2 for the remainder of the test material composition) in water. In both experiments, applications of approximately 20 mg/cm^2 were applied to the skin. Skin discs of 1.0 cm^2 were exposed to the test substance for 30 minutes, and then rinsed. The amount of the test substance systemically available from the direct dye cream and the aqueous solution was 1.96 ± 0.83 µg/cm^2 and 6.52 ± 3.58 µg/cm^2, respectively.

Human

Ten males had 20 µL of the test substance, 1 mM test material (55.5% Basic Red 76; see Table 2 for the remainder of the test material composition) in 40% aqueous isopropanol, applied to five separate areas (5.3 cm^2) of the inner forearm.[3] The dye stains were removed by ten repeated strippings with tape after 10 minutes, 24, 48, and 72 hours, and the amount of dye that potentially penetrated was estimated. The dye was not suspected to have been diffused into the horny layer, and the researchers concluded that the dye was not absorbed by the skin.

Absorption, Distribution, Metabolism, and Excretion (ADME)

Animal

Dermal

An application of 200 µL of a hair setting lotion containing 0.1% test material (55.5% ^{14}C-labelled Basic Red 76 (labeling regiochemistry not stated); see Table 2 for the remainder of the test material composition) was applied to the skin of 3 Sprague-Dawley rats over an area of 1.5" x 1.5", corresponding to an exposure of 31.3 mg/cm^2 skin.[3] The duration of exposure was not reported. Radioactivity recovered from feces and urine was less than 0.2% and 0.3% of the applied dose, respectively. A maximum total absorption was calculated to be 0.5%, corresponding to a maximum of 0.15 µg/cm^2 of skin. Excretion of radioactivity in urine and feces was measured for 24 hours after application. The amount of radioactivity recovered in the carcass or organs was not determined.

Other studies were performed using a setting lotion and shampoo formulation containing 0.2 and 0.5% test material (55.5% ^{14}C-labelled Basic Red 76 (labeling regiochemistry not stated); see Table 2 for the remainder of the test material composition), respectively.[3] Application was performed on the clipped (but not shaven) skin of Wistar rats. After an application of 100 µL to 5 Wistar rats/sex, treatment sites were covered with a non-occlusive glass capsule containing small holes. Exposure occurred for 24 hours. In rats dosed with the setting lotion formulation containing 0.2% (25 µg of test material/cm^2 skin), more than 80% of the applied radioactivity was recovered on the hair, and about 10% on the skin. The radioactivity recovered in the urine and feces was 0.07 and 0.16%, respectively. No radioactivity was detected in the carcasses. In a different study where rats were exposed for 24 hours to 70 and 140 µL of a shampoo containing 0.5% test material (55.5% ^{14}C-labelled Basic Red 76 (labeling regiochemistry not stated); see Table 2 for the remainder of the test material composition), $\geq$ 93% of the applied radioactivity was recovered in the hair rinsings. Approximately 2.1 and 1.7% of the radioactivity was recovered on the treated skin. The radioactivity recovered in the urine was less than 0.007% in males and 0.002% in females. Less than 10% of the applied radioactivity was observed in the feces of treated animals. The amount of radioactivity recovered in the carcass or organs was not determined.

Parenteral

Three male Wistar rats were given a single intravenous (i.v.) dose of 2.5 mg/kg bw of the test material (55.5% ^{14}C-labelled Basic Red 76 (labeling regiochemistry not stated); see Table 2 for the remainder of the test material composition) in physiological saline.[3] Approximately 63 and 15% of the administered test substance was recovered in the feces and urine, respectively. The level of radioactivity detected in the carcass 24 hours after the administrated dose was approximately 9%. In a similar study, mice were given a single subcutaneous dose of 5 mg/kg of the same test substance. Two minutes after administration, 31% of the radioactivity was present in the liver and kidneys, 9% in the small intestine, and 1.3% in the lungs. After 24 hours, the total radioactivity in the liver, kidneys and lungs decreased to 33.7% of the given dose. Specific radioactivity was highest in the cecum and large intestine by the end of the study.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

In an acute toxicity study, 3 CF1 mice were treated with a single oral dose of 1, 2.51, or 5.01 g/kg bw test material (55.5% Basic Red 76; see Table 2 for the remainder of the test material composition) in a volume of 20 to 40 mL/kg, and 10 male mice received the top dose of 10 mg/kg bw (method of oral dosing was not provided).[3] The animals were observed for 14 days after treatment. Lethargy and breathing disorders were observed in mice given 10 g/kg bw of the test substance. The LD_{50} value was reported to be >10 g/kg bw.

Wistar rats (3/sex) were given a single oral dose of 2 g/kg bw test material (62% Basic Red 76; see Table 2 for the remainder of the test material composition) in propylene glycol via gavage.[2,12] Three male and one female rat displayed hunched

posture on the first day of treatment. Red staining of the back and/or snout and/or head was observed in one female and two male mice. Red and/or yellow feces and/or urine were seen in all animals. The established oral LD_{50} value was > 2 g/kg bw.

CFY rats (2/sex/group) were given a single oral dose (0, 0.1, 1, 4, 8, or 16 g/kg bw) of the test material (55.5% Basic Red 76; see Table 2 for the remainder of the test material composition) in 1% aqueous methylcellulose (method of oral dosing was not provided).[3] Animals were observed for 14 days after treatment. All animals survived treatment. Lethargy, piloerection, decreased respiratory rate, and hunched posture were observed. At the 8 and 16 g/kg bw dose levels, red staining of the urine and feces was noted. The acute lethal dose of the test substance was reported to be > 16 g/kg bw.

Short-Term Toxicity Studies

Wistar MuRa Han 67 SPF rats (20/sex/group) were given 0 or 200 mg/kg bw of the test material (55.5% Basic Red 76; see Table 2 for the remainder of the test material composition) in a volume of 10 mL/kg water via gavage 5 days per week for 12 weeks.[3] All animals survived the duration of the experiment. Aggressive behavior was apparent in all dosed animals. In males, body weight gain was similar to the control group, however, in females, slight but significantly lower mean body weights were recorded (95 - 96% of control) on the 5th, 7th, 9th, and 12th week, and at the end of the study. Colored urine was observed in all dosed animals. Increases in the mean cell volume and hematocrit values were noted in male rats and some female rats. In male rats, a slight increase in cerebral weights compared to the control groups was observed. In females, kidney, heart, and liver weights were lower than those of control animals. The no-observable-adverse-effect-level (NOAEL) was reported to be < 200 mg/kg bw/d.

Subchronic Toxicity Studies

Groups of 10 female and 10 male Sprague-Dawley CD rats were given the test material (55.5% Basic Red 76; see Table 2 for the remainder of the test material composition), in amounts of 0 and 20 mg/kg bw in a volume of 10 mL/kg aqueous solution. Treatments were given 5 days/week for 13 weeks by gavage.[3] No mortalities were reported. Body weight gain was similar in the control and treated groups. No other effects were noted. The dose of 20 mg/kg bw/d was determined to be a "no effect level."

SPF-bred Wistar rats (12/sex/group) were given a single daily dose of the test material (80.5% Basic Red 76; see Table 2 for the remainder of the test material composition) in distilled water via gavage for 90 days.[2,13] Rats received doses of 0, 60, 250, or 1000 mg/kg bw/d. One female dosed with 60 mg/kg bw/d was found dead on day 60, however a gavage error was considered to be the cause of death. Staining of body parts and discoloration of the feces/urine was observed. Infrequent and intermittent clonic spasms were observed in some test animals in all dose groups. No relevant body weight or food intake level changes were noted. Destruction of red blood cells, increased tissue iron in the spleen and liver, and increased serum bilirubin levels were noted in animals dosed with 250 and 1000 mg/kg bw/d. Thyroid follicular cell hypertrophy and adenohypophyseal cell hypertrophy was observed in rats given 1000 mg/kg bw/d. At 60 mg/kg bw/day, decreased red blood count cells, hemoglobin levels, hematocrit levels, and mean corpuscular hemoglobin concentrations were seen. The NOAEL was reported to be 60 mg/kg/d.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Doses of 0, 60, 250, and 1000 mg/kg bw/d of the test substance (89.1% Basic Red 76; see Table 2 for the remainder of the test material composition) were given via gavage to Wistar rats (24/group) on days 6 to 20 of gestation.[2,14] All females were killed and examined 21 days after mating. In rats treated with 60 mg/kg bw/d, no signs of reproductive toxicity were observed. At the 250 mg/kg bw/d dose level, decreased body weight, weight gain, and food consumption was observed in maternal rats. A decrease in fetal body weight was also reported. Similar findings were seen in rats treated with 1000 mg/kg bw/d, however, signs of toxicity were more pronounced in the group treated with 250 mg/kg bw/d. Slight increases in the thinning of the central tendon region of the diaphragm and left-sided umbilical artery was observed. The maternal and developmental NOAEL was determined to be 60 mg/kg bw/d. A similar study was performed using Sprague Dawley CD rats.[3] Rats were given the test substance (55.5% Basic Red 76; see Table 2 for the remainder of the test material composition) in distilled water in doses of either 0 (20 rats) or 50 mg/kg (25 rats) bw/d on days 6 - 15 of gestation via gavage. On day 20 of gestation, the dams were killed. No adverse effects were reported in dams or fetuses treated with up to 50 mg/kg bw/d.

GENOTOXICITY

Details of the genotoxicity studies summarized below are provided in Table 4. Information regarding the test material composition in these studies can be observed in Table 2.

In Vitro

Basic Red 76 was generally not genotoxic. Negative results were observed in Ames tests at doses of up to 5000 µg/plate, with or without metabolic activation.[2,3,3,3,15] Positive and negative results were observed in an Ames test using *Salmonella typhimurium*. Negative results were obtained in mammalian gene mutation assays using Chinese hamster V79 cells (dose not specified) and mouse lymphoma L5178Y cells at doses up to 318 µg/mL with and without metabolic activation or 425 µg/mL without metabolic activation.[3,16] The test substance was not genotoxic in a mammalian chromosome aberration assay using Chinese hamster V79 cells at doses of up to 500 mg/mL, but it was clastogenic at a dose of 1000 mg/mL without metabolic activation.[3] The test substance was clastogenic in an in vitro micronucleus test in V79 cells at doses of up to 1700 µg/mL with metabolic activation, and in doses of 212.5 µg/mL and higher without metabolic activation.[16] However, in in vivo micronucleus assays in mice, the test substance was not clastogenic at doses of up to 5000 mg/kg given orally to mice.[3,17]

CARCINOGENICITY

Information regarding the carcinogenicity of Basic Red 76 was not found, however the potential metabolites of Basic Red 76 have induced a number of multi-organ tumors in several studies.[10] Both malignant and benign tumors in the bladder, spleen, subcutaneous tissues, kidneys, adrenal gland, liver, mammary glands, skin, blood, blood vessels, thyroid, lungs, gallbladder and renal pelvis, have been associated with the exposure of the metabolized aromatic amines.

DERMAL IRRITATION AND SENSITIZATION

Irritation

Animal

A skin irritation test was performed according to OECD TG 404.[2,18] A semi-occlusive patch containing 500 mg of the test substance (62% Basic Red 76; see Table 2 for the remainder of the test material composition) was applied to approximately 150 cm^2 of shaved skin of 3 New Zealand White rabbits. Patches were removed after 4 hours. Skin was scored 1, 24, 48 and 72 hours, as well as 7, 10 and 14 days after removal of dressing. No visible signs of irritation were observed. Minimal red staining was noted in all animals.

In another study, a 24-h occlusive patch of 0.5 g undiluted test material (62% Basic Red 76; see Table 2 for the remainder of the test material composition) was applied to a 1 sq. in area of intact or scarified skin of the back of 3 New Zealand White rabbits.[3] No reactions were reported. A similar study was performed with a diluted solution of the test substance. A dose of 0.5 g of the test material (62% Basic Red 76; see Table 2 for the remainder of the test material composition) was dampened with 0.5 mL distilled water and applied to a 1 sq. in area of intact or scarified skin of the back of 3 New Zealand White rabbits. No reactions were recorded.

Sensitization

Animal

Twenty-five microliters of the test material (62% Basic Red 76; see Table 2 for the remainder of the test material composition) was applied in concentrations of 2.5, 5, and 10% in a 7:3 v/v ethanol:water mixture.[2,19] Applications were made on the earlobes of mice (4 females/group) in a local lymph node assay (LLNA). The test substance was applied once daily for 3 days. Five days after the first treatment, mice were given an intravenous injection of radio-labelled thymidine. Mice were killed 5 hours after thymidine administration. The draining lymph nodes were excised, pooled, placed in scintillation vials, and tested for proliferative capacity. The stimulation index values were 0.9, 1.1, and 1.3, for the 2.5, 5, and 10% dose levels, respectively. The test material was considered to be non-sensitizing.

In a Magnusson-Kligman test performed according to OECD TG 406, the sensitization potential of the test material (55.5% Basic Red 76; see Table 2 for the remainder of the test material composition) was evaluated using 10 female Hartley/Dunkin guinea pigs.[3] Three injections were given before patch testing. The first consisted of the material solution (0.1% test material in water), the second, Freund's Complete Adjuvant (FCA) diluted with an equal volume of water, and the third, a 1:1 mixture of the material solution and FCA. The induction of sensitization was made through 3 pairs of 2 intradermal injections. One week after the administration of injections, a solution of 75% w/v of the test substance in distilled water was applied to the skin. A challenge patch was applied 2 weeks later using the test substance at a concentration of 25% w/v. Irritation was noted after administration of the intradermal injection and topical application. Half of the test animals displayed erythema after the challenge phase that was resolved by 48 hours.

OCULAR IRRITATION STUDIES

Animal

An ocular irritation test was performed according to OECD TG 405.[2,20] The test material (62% Basic Red 76; see Table 2 for the remainder of the test material composition) was instilled at an amount of 0.1 g into the conjunctival sac of 3 New Zealand White rabbits. Treated eyes were rinsed following a 24 hour exposure period. Scoring occurred 1, 24, 48, and 72 hours, as well as 7 days after instillation. Redness of the conjunctivae and sclerae, discharge, and chemosis were apparent at the beginning of treatment, but were no longer present after 72 hours. Minimal staining of the eyes was observed after 1 hour and 24 hours in all subjects. Staining was present in two animals at the 48 hour mark, and in one animal at the 72 hour mark. No abnormalities or corrosion was reported in the cornea or iris of test animals.

In a similar study using 3 New Zealand White rabbits, the test material (55.5% Basic Red 76; see Table 2 for the remainder of the test material composition) in physiological saline was instilled into the conjunctival sac (0.1 mL) of one eye of each rabbit.[3] The concentration of the test substance used was 0.5%. Eye reactions were recorded after 30 and 60 minutes, and 24 and 48 hours. No effects on the cornea or iris were reported, however, discoloration was noted.

SUMMARY

Basic Red 76 is a monoazo color that functions as a hair colorant and hair-conditioning agent. Synthesis of the dye includes diazotization of a primary aromatic amine in a cold, aqueous, acidic solution with sodium nitrite. The resulting diazonium salt is highly reactive and a arylazo-dehydrogenation reaction with an aromatic alcohol quickly results in an azo dye.

According to the 2018 VCRP survey data, Basic Red 76 is reported to be used in 46 hair coloring formulations. The results of the concentration of use survey conducted by the Council indicate that the highest concentration of use reported for Basic Red 76 was 0.35% in hair dyes and colors. Basic Red 76 is listed in the EU Cosmetics Regulation 1223/2009 Annex III, and is allowed in non-oxidative hair dye products at a maximum concentration of 2.0%.

In a dermal absorption study involving pig skin, 2% test material (80.5% Basic Red 76) was applied to samples in either a direct dye cream lotion or aqueous formulation. Applications were performed in amounts of approximately 20 mg/cm^2. The amount of the test material systemically available from the direct dye cream and the aqueous solution was 1.96 ± 0.83 µg/cm^2 and 6.52 ± 3.58 µg/cm^2, respectively. In a human study, 20 µL of 1 mM of the test substance (55.5% Basic Red 76; see Table 2 for the remainder of the test material composition) in aqueous isopropanol was applied to the forearm. The dye stains were removed by ten repeated strippings with tape after 10 minutes, 24, 48, and 72 hours. The dye was not suspected to have been diffused into the horny layer.

When an application of 200 µL of a hair setting lotion containing 0.1% test material (55.5% ^{14}C-labelled Basic Red 76) was applied to the skin of rats over an area of 1.5" x 1.5". The amount of radioactivity recovered from feces and urine (measured for 24 hours after application) was less than 0.2% and 0.3% of the applied dose, respectively. A maximum total absorption was calculated to be 0.5%, corresponding to a maximum of 0.15 µg/cm^2 of skin. Other studies were performed in which a setting lotion and shampoo formulation containing 0.2 and 0.5% test material (55.5% ^{14}C-labelled Basic Red 76), respectively, were applied to the skin of Wistar rats for 24 hours. In rats dosed with 0.2% test material, over 80% of the applied radioactivity was recovered on the hair. The radioactivity recovered in the urine and feces was 0.07 and 0.16%, respectively. In rats treated with the formulation containing 0.5% test material, 93-102% of the applied radioactivity was recovered in hair rinsings. Approximately 2.1 and 1.7% radioactivity was recovered on the skin treated with 70 and 140 µL of the shampoo, respectively. The radioactivity recovered in the urine was < 0.007% in males and 0.002% in females. Less than 10% of the applied radioactivity was observed in the feces of treated animals. Three male Wistar rats were given a single i.v. dose of 2.5 mg/kg bw of test material (55.5% ^{14}C-labelled Basic Red 76) in physiological saline, intravenously. Approximately 63 and 15% of the administered dose was recovered in the feces and urine, respectively. In a similar study, mice were given a single subcutaneous dose of 5 mg/kg of the same test substance. Two minutes after administration, 31% radioactivity was present in the liver and kidneys, 9% in the small intestine, and 1.4% in the lungs. After 24 hours, the total radioactivity in the liver, kidneys and lungs decreased to 33.7% of the given dose.

The oral LD_{50} of a test substance containing Basic Red 76 was > 10 g/kg bw (55.5% Basic Red 76) in CF1 mice, > 2 g/kg bw (62% Basic Red 76) in Wistar rats and > 16 g/kg (55.5% Basic Red 76) bw in CFY rats. These values were the highest doses tested in each study. Some signs of toxicity were observed.

The NOAEL was reported to be less than 200 mg/kg bw/d in Wistar MuRa Han 67 SPF rats dosed via gavage 5 days a week for 12 weeks (test material (55.5% Basic Red 76)). The dose of 20 mg/kg bw/d was determined to be a no effect level in

Sprague-Dawley CD rats dosed by gavage for 5 days/week for 13 weeks (test material (55.5% Basic Red 76)). Toxic effects included lowered body and organ weights. Basic Red 76-induced toxic effects were noted in Wistar rats dosed orally for 90 days with as low as 250 mg/kg bw/d (test material (80.5% Basic Red 76)).

No signs of reproductive toxicity were observed in Wistar rats given 60 mg/kg bw/d via gavage (test material (89.1% Basic Red 76)); however toxic effects were noted at the 250 mg/kg bw/d dose level and higher when the same test substance was used. The maternal and developmental NOAEL was determined to be 60 mg/kg bw/d. In a different study, no adverse effects were reported in Sprague-Dawley CD rats given up to 50 mg/kg bw/d via gavage on gestation days 6-15 (test substance (55.5% Basic Red 76)).

Basic Red 67 were not genotoxic in Ames tests and mammalian gene mutation assays using Chinese hamster V79 cells and mouse lymphoma L5178Y cells, with and without metabolic activation. A chromosomal aberration assay using Chinese hamster V79 cells yielded negative results at up to 500 mg/mL; however, at the 1000 mg/mL dose level, without metabolic activation, Basic Red 76 was clastogenic. An in vitro micronucleus test in V79 cells yielded positive results at doses up of 1700 µg/mL, with metabolic activation, and at doses of 212.5 1700 µg/mL and higher, without metabolic activation. Negative results were observed in in vivo micronucleus assays in mice at up to 5000 mg/kg.

The potential metabolites of Basic Red 76 have induced a number of multi-organ tumors in several studies. Both malignant and benign tumors in the bladder, spleen, subcutaneous tissues, kidneys, adrenal gland, liver, mammary glands, skin, blood, blood vessels, thyroid, lungs, gallbladder and renal pelvis, have been associated with the exposure of the metabolized aromatic amines.

No irritation was reported when New Zealand White rabbits were dermally dosed with 500 mg or 0.5 g/in^2 of the test substance (62% Basic Red 76) under an occlusive patch. Basic Red 76 was considered a non-sensitizer when 25 µL of the test substance (62% Basic Red 76) was applied to mouse earlobes at a concentration of up to 10%. Erythema that was resolved after 48 hours was observed after the challenge phase in Hartley/Dunkin guinea pigs dosed with 75% (induction) and 25% (challenge) test material.

Ocular irritation was observed in New Zealand White rabbits following instillation of 0.1 g of the test material (62% Basic Red 76) into the conjunctival sac; this effect was resolved within 72 hours. In a different study, no irritation was reported when the test substance (55.5% Basic Red 76), at a concentration of 0.5%, in physiological saline, was placed in the conjunctival sac of New Zealand White rabbits.

DATA NEEDS

CIR is seeking any additional data on Basic Red 76 that would help the CIR Expert Panel assess the safety of this ingredient as it is used in cosmetics. Particularly, information on the composition of Basic Red 76 as used is cosmetics is requested.

TABLES

Table 1. Physical and Chemical Properties[2]

Property	Value
Physical Form	fine powder
Color	red
Molecular Weight (g/mol)	371.86
Melting Point (°C)	200
Water Solubility (g/L @ room temperature)	10 - 100
Ethanol Solubility (g/L @ room temperature)	0.3 - 3
DMSO Solubility (g/L @ room temperature)	1 - 10
log P_{ow}	-1.7834 ± 0.1131
Ultraviolet absorption (nm)	235, 332, 503

Table 2. Impurities/Components

Basic Red 76 (% w/w)	62[2]	80.5[2]	89.1[2]	55.5 (as chloride)[3]
Water (% w/w)	5.1	4.1	3.1	NR
Monomethyl sulphate (% w/w)	11.8	15.9	11.4	NR
o-anisidine (%)	0.0005	0.0019	0.0011	NR
Chloromethane (% w/w)	0	0.3	0.1	NR
Methyl acetate (% w/w)	0	0.1	NR	NR
Methyl formate (% w/w)	0	0.4	NR	NR
7-Hydroxy-*N*,*N*,*N*-trimethylnaphthalen-2-aminium chloride (%)	0	< 0.05	< 500 ppm	NR
Methanol (% w/w)	0	0	0.7	NR
Sulphated ash (% w/w)	0.4	0.3	0.1	NR
Chloride (% w/w)	1.6	2.7	4.4	NR
Sodium (%)	0.063	0.019	0.024	NR
Calcium (%)	0.059	0	NR	NR
Saccharose (% w/w)	25.8	0	NR	NR
Sugar (undefined (%))	NR	NR	NR	16
Volatile matter/water of crystallization (undefined (%))	NR	NR	NR	14
Inorganic salts – chloride, sulphate, etc. (undefined (%))	NR	NR	NR	up to 100%

NR = Not Reported

Table 3. Frequency (2018) and Concentration of Use (2017) of Basic Red 76, specific to hair coloring categories

	# of Uses[5]	Conc of Use (%)[6]
Totals*	**46**	**0.057 - 0.35**
Exposure Type		
Hair Dyes and Colors	4	0.057 – 0.35
Hair Tints	3	0.18
Hair Shampoos (coloring)	11	0.2
Other Hair Coloring Preparations	5	0.13
Hair Rinses (Coloring)	23	NR

NR = no reported use

Table 4. **Genotoxicity studies**

Test Article	Concentration/Dose	Vehicle	Test System/Organism	Procedure	Results	Reference
IN VITRO						
80.5% Basic Red 76; see Table 2 for the remainder of the test material composition	Experiment 1: 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate Experiment 1: 33, 100, 333, 1000, 2500 and 5000 µg/plate	DMSO	*Salmonella typhimurium* (TA98, TA100, TA102, TA1535, and TA1537)	Bacterial reverse mutation assay; Experiment 1 involved direct plate incorporation with a 48 hour incubation time with and without S9 mix. Experiment 2 involved the same dosing, excluding the 10 µg/plate level. Cells were pre-incubated for 60 minutes and at least 48 hours of incubation with and without S9 mix.	Non-mutagenic	[2,15]
55.5% Basic Red 76; see Table 2 for the remainder of the test material composition	NR	NR	*S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	Bacterial reverse mutation assay; with and without metabolic activation.	The test substance induced mutation in TA1537 with metabolic activation and in TA1538 with and without metabolic activation.	[3]
80.5% Basic Red 76; see Table 2 for the remainder of the test material composition	Experiment 1: 26.6, 53.1, 106.3, 212.5, 318.8 µg/ml Experiment 2: 53.1, 106.3, 212.5, 318.8, 425 µg/ml	Deionized water	Mouse lymphoma cell line L5178Y	Mammalian cell gene mutation assay; Experiment 1: with and without S9-mix; treated for 4 hours; expression period of 72 hours Experiment 2: without S9-mix; treated for 24 hours; expression period of 48 hours A pre-test treatment of up to 1700 µg/ml with and without metabolic activation was also performed.	Non-mutagenic	[2,21]
55.5% Basic Red 76; see Table 2 for the remainder of the test material composition	NR	NR	Chinese hamster V79 cells	Mammalian cell gene mutation assay; V79 cells were treated with Basic Red 76 with and without metabolic activation	The test substance did not induce gene mutation in the V79 cells.	[3]
55.5% Basic Red 76; see Table 2 for the remainder of the test material composition	Experiment 1 (part 1): 62.5, 125, 250 mg/mL Experiment 1 (part 2): 250, 500, 1000 mg/mL Experiment 1 (part 3): 50, 100, 200 mg/mL Experiment 2 (part 1): 62.5, 125, 250 mg/mL Experiment 2 (part 2): 31.3, 62.5, 250 mg/mL	NR	Chinese hamster lung V79 cells	Mammalian chromosome aberration test; Experiment 1 (part 1): cells treated in absence of S9 for 18 h Experiment 1 (part 2): cells treated in absence of S9 for 28 h Experiment 2(part 3): cells treated in absence of S9 for 18 h Experiment 2 (part 1 and 2): cells treated in presence of S9 (duration not stated)	Non clastogenic at the 62.5, 125, 250, and 500 mg/mL levels; Clastogenic at the 1000 mg/mL level; a slight but significant increase in the frequency of chromosomal aberrations was present.	[3]
80.5% Basic Red 76; see Table 2 for the remainder of the test material composition	Experiment 1 (part 1): 53.1, 106.3, 212.5 µg/ml Experiment 1 (part 2): 150, 200, 300 µg/ml Experiment 2 (part 1): 106.3, 212.5, 425, 850, 1700 µg/ml Experiment 2 (part 2): 106.3, 212.5, 425 µg/ml	Deionized water	Chinese hamster V79 Cells	Micronucleus test; Experiment 1 (part 1): with and without S9-mix, treated for 4 hours; harvest time 24 hours after beginning of treatment Experiment 1 (part 2): with S9-mix, treated for 4 hours; harvest time 24 hours after beginning of treatment Experiment 2 (part 1): without S9-mix; treated for 20 hours, harvest time 24 hours after beginning of treatment Experiment 2 (part 2): with S-9 mix; treated for 4 hours, harvest time 48 hours after the beginning of treatment	Clastogenic In experiment 1, without metabolic activation, increases in cells with micronuclei were not noted. In experiment 1 (part 1), without metabolic activation, a biologically relevant increase in cells with micronuclei was not observed Dose-dependent, biologically relevant increases in micronuclei were observed in experiment 2 with and without metabolic activation.	[2,16]

Table 4. **Genotoxicity studies**

Test Article	Concentration/Dose	Vehicle	Test System/Organism	Procedure	Results	Reference
IN VIVO						
80.5% Basic Red 76; see Table 2 for the remainder of the test material composition	0, 25, 50, 100 mg/kg bw (oral); 200 mg/kg bw (intraperitoneal)	Deionized water	NMRI mice (5 mice/sex/group)	Micronucleus test; Mice were given oral doses or intraperitoneal doses of Basic Red 76. Toxicity was determined by measuring the ration of PCE and TE. Duration of dosing was not provided.	Non- clastogenic	[2,17]
55.5% Basic Red 76; see Table 2 for the remainder of the test material composition	5000 mg/kg bw	NR	CFW 1 mice (5/sex/group)	Micronucleus test; Mice were given doses of Basic Red 76 via gavage. Duration of dosing was not provided.	Non-clastogenic	[3]

NR = Not Reported; DMSO = dimethyl sulfoxide, PCE = polychromatic erythrocyte; TE = total erythrocytes

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