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## **Safety Assessment of Basic Red 76 as Used in Cosmetics**

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## **ABSTRACT**

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) assessed the safety of Basic Red 76, which is reported to function in cosmetics as a hair colorant and hair-conditioning agent. The Panel reviewed the available data to determine the safety of this ingredient. The Panel concluded that Basic Red 76 is safe for use as a hair dye ingredient in the present practices of use and concentration described in the safety assessment.

## **INTRODUCTION**

This is a safety assessment of Basic Red 76 as used as a hair dye ingredient. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (WINCI; *Dictionary*), Basic Red 76 is a monoazo color that functions as a hair colorant and hair-conditioning agent.<sup>1</sup> However, according to the US Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP) database, this ingredient is reported to be used in nail products.<sup>2</sup> Although Basic Red 76 is exempt for use as hair dye, use in nail products is not an approved use for this cosmetic ingredient, and therefore, the Panel is not reviewing the safety for such use.

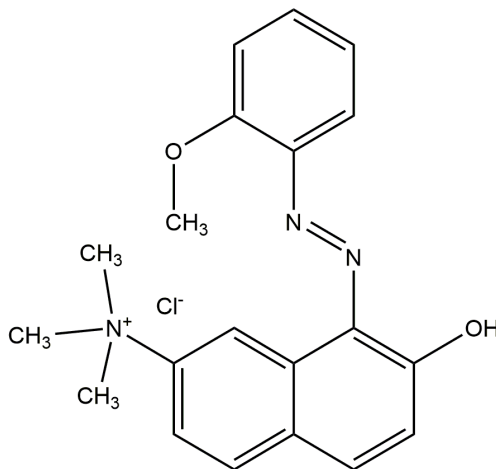
This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that CIR typically evaluates, is provided on the CIR website (<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 Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Much of the data included in this safety assessment was gathered from the opinions of European scientific committees, specifically, the Scientific Committee on Consumer Safety (SCCS)<sup>3</sup> and Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP).<sup>4</sup>

## **CHEMISTRY**

### **Definition and Structure**

As given in the *Dictionary*, Basic Red 76 (CAS No. 68391-30-0) is the azo dye that conforms to the following structure<sup>1</sup>:



**Figure 1. The azo dye, Basic Red 76**

### **Physical and Chemical Properties**

Basic Red 76 is a cationic direct dye that is water soluble.<sup>3</sup> Ultraviolet-visible (UV-Vis)-spectra showed maxima at 235 nm (62% Basic Red 76) and 332 nm (80% Basic Red 76). This ingredient, in its pure form, is a red powder with a melting point of 200°C and an octanol-water partitioning coefficient of -1.78. A list of chemical and physical properties for Basic Red 76 is provided in Table 1.

### **Method of Manufacture**

While no methods were found in the publicly available literature specific to the preparation of Basic Red 76, most azo dyes are synthesized in the same manner.<sup>5</sup> 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.<sup>3</sup> 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. Throughout the report, please refer to Table 2 to note the full compositions of the test materials. In addition, Table 2 also provides composition information of Basic Red 76 as used in the market.

### **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 2019 VCRP survey data, Basic Red 76 is reported to be used in 48 hair-coloring formulations and 2 nail polish and enamel formulations.<sup>6</sup> 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.<sup>7</sup> Concentration of use data were not provided for use in nail products. Detailed data regarding concentration and frequency of use can be reviewed in Table 3.

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 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 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.<sup>8</sup> These authors concluded that, in its present form, the hair dye self-test has severe limitations. 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, stating 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.<sup>9</sup>

Additionally, according to a report published in 2018, a different method of patch-testing was suggested utilizing more relevant protocols regarding exposure time and test preparation that reflect the actual use conditions with hair dying.<sup>10</sup> According to this study, a self-test protocol for an allergy alert test (AAT) was developed that elicits a self-noticeable alert signal in *p*-phenylenediamine (PPD)-allergic consumers after a 45 minute exposure to the hair dye mixed with developer.

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%.<sup>11</sup> According to the SCCS, Basic Red 76 containing up to 18% 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%.<sup>3</sup>

### **TOXICOKINETIC STUDIES**

Azo bond cleavage and reduction is mediated by enzymes found in the liver, skin, and intestines.<sup>12</sup> Responsible cofactors and 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.<sup>3,13</sup> 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) in direct dye was applied to skin samples. Experiment B involved 2% test material (80.5% Basic Red 76) in water. In both experiments, applications of approximately 20 mg/cm<sup>2</sup> were applied to the skin. Skin discs of 1.0 cm<sup>2</sup> were exposed to the test substance for 30 minutes, and then rinsed. The receptor fluid used was a phosphate buffered saline. Within 48 hours after application, the percent of applied dose of Basic Red 76 recovered in the stratum corneum, epidermis/dermis, and receptor fluid were 1.44%, 0.046%, and 0.0049%, respectively. Samples treated with the aqueous solution displayed penetration amounts of 3.87% in the stratum corneum, 1.77% in the epidermis/dermis, and 0.012% in the receptor fluid. The amount of the test substance that was considered bioavailable from the direct dye cream and the aqueous solution was  $1.96 \pm 0.83 \mu\text{g}/\text{cm}^2$  and  $6.52 \pm 3.58 \mu\text{g}/\text{cm}^2$ , respectively.

### **Human**

Ten male subjects had 20  $\mu\text{L}$  of a 1 mM test material (55.5% Basic Red 76), in 40% aqueous isopropanol, applied to five separate areas (5.3 cm<sup>2</sup>) of the inner forearm.<sup>4</sup> 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. No other information regarding this study was provided.

## **Absorption, Distribution, Metabolism, and Excretion (ADME)**

### **Animal**

#### **Dermal**

An application of 200  $\mu\text{L}$  of a hair setting lotion containing 0.1% test material (55.5% [<sup>14</sup>C]-labelled Basic Red 76, labeling regiochemistry not stated) 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<sup>2</sup> skin (and 31.3  $\mu\text{g}$  Basic Red 76/cm<sup>2</sup> skin).<sup>4</sup> The rats were anesthetized for 1 hour after application, then they were fitted with a collar to prevent licking. 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  $\mu\text{g}/\text{cm}^2$  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% [<sup>14</sup>C]-labelled Basic Red 76, labeling regiochemistry not stated), respectively.<sup>4</sup> Application was performed on the clipped (but not shaven) skin of Wistar rats. After an application of 100  $\mu\text{L}$  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% of the test material (25  $\mu\text{g}$  of test material/cm<sup>2</sup> skin), more than 80% of the applied radioactivity was recovered on the hair, and about 10% was recovered 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  $\mu\text{L}$  of a shampoo containing 0.5% test material (55.5% [<sup>14</sup>C]-labelled Basic Red 76, labeling regiochemistry not stated),  $\geq 93\%$  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 in males and females, respectively. 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% [<sup>14</sup>C]-labelled Basic Red 76, labeling regiochemistry not stated) in physiological saline.<sup>4</sup> Approximately 63 and 15% of the administered test substance was recovered in the feces and urine, respectively, over a duration of 24 hours. The level of radioactivity detected in the carcass 24 hours after the administered dose was approximately 9%. In another 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**

#### **Oral**

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) 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).<sup>4</sup> The animals were observed for 7 days after treatment. Lethargy and breathing disorders were observed in mice given 10 g/kg bw of the test substance. The LD<sub>50</sub> 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) in propylene glycol via gavage.<sup>3,14</sup> 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<sub>50</sub> 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) in 1% aqueous methylcellulose (method of oral dosing was not provided).<sup>4</sup> 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**

#### **Oral**

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) in a volume of 10 mL/kg water via gavage 5 days per week for 12 weeks in a screening study.<sup>4</sup> 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 5<sup>th</sup>, 7<sup>th</sup>, 9<sup>th</sup>, and 12<sup>th</sup> 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, as 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**

#### **Oral**

Groups of 10 female and 10 male Sprague-Dawley CD rats were dosed with 0 and 20 mg/kg bw of the test material (55.5% Basic Red 76), in a volume of 10 mL/kg aqueous solution.<sup>4</sup> The test article was administered 5 days/week for 13 weeks by gavage. 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) in distilled water via gavage for 90 days.<sup>3,15</sup> 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; however, this effect is not considered relevant to humans as rats have a significantly higher sensitivity to this effect. 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. This study also included recovery groups of 5 rats/sex/dose group. The animals in these groups were examined after treatment, for four weeks. The hematological effects observed in the treated animals were widely resolved during the 28-day recovery period.

## **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) were given via gavage to Wistar rats (24/group) on days 6 to 20 of gestation.<sup>3,16</sup> All females were killed and examined 21 days after mating. In rats treated with 60 mg/kg bw/d, no signs of developmental 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 in offspring. The maternal and developmental NOAEL was determined to be 60 mg/kg bw/d. Another study was performed using Sprague-Dawley CD rats.<sup>4</sup> Rats were given the test substance (55.5% Basic Red 76) 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 50 mg/kg bw/d.

### **GENOTOXICITY**

Details of the genotoxicity studies summarized below are provided in Table 4. The percent test material provided in Table 4 corresponds to the test material described in more detail in Table 2.

Basic Red 76 was generally not genotoxic. Negative results were observed in Ames tests at concentrations of up to 5000 µg/plate, with or without metabolic activation (*Salmonella typhimurium* TA98, TA100, TA102, TA1535, and TA1537).<sup>3,4</sup> However, in another study, positive results were observed in an Ames test using *S. typhimurium* in strains TA1537 and TA1538 (test concentration not specified), while negative results were observed in strains TA98, TA100, and TA1535 in concentrations as high as 5000 µg/plate.<sup>3</sup> 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, and 425 µg/mL, with and without metabolic activation.<sup>4,17</sup> The test substance was not genotoxic in a mammalian chromosome aberration assay using Chinese hamster V79 cells at concentrations of up to 500 mg/mL, but it was clastogenic at a dose of 1000 mg/mL without metabolic activation.<sup>4</sup> The test substance was clastogenic in an in vitro micronucleus test in V79 cells at concentrations as low as 212.5 µg/mL with and without metabolic activation.<sup>17</sup> Positive results were observed mainly at concentrations associated with test material precipitation. In in vivo micronucleus assays in mice, the test substance was not clastogenic at concentrations of up to 5000 mg/kg given orally to mice.<sup>4,18</sup>

### **CARCINOGENICITY**

Information regarding the carcinogenicity of Basic Red 76 was not found, however the potential metabolites of Basic Red 76, such as *o*-anisidine, have induced a number of multi-organ tumors according to third party summaries of animal studies cited by National Industrial Chemicals Notification and Assessment Scheme (NICNAS).<sup>12</sup> 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. NICNAS, however, did not provide any specific evidence stating that Basic Red 76 is metabolized to *o*-anisidine.

### **DERMAL IRRITATION AND SENSITIZATION**

#### **Irritation**

##### **Animal**

A skin irritation test was performed according to OECD TG 404.<sup>3,19</sup> A semi-occlusive patch containing 500 mg of the test substance (62% Basic Red 76) was applied to approximately 150 cm<sup>2</sup> of shaved skin of 3 New Zealand White rabbits. Patches were removed after 4 hours. Skin was evaluated 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) was applied to a 1 in<sup>2</sup> of intact or scarified skin of the back of 3 New Zealand White rabbits.<sup>4</sup> No reactions were reported. A similar study was performed with the same test substance that was dampened for adhesion. A dose of 0.5 g of the test material (62% Basic Red 76) was dampened with 0.5 mL distilled water and applied to a 1 in<sup>2</sup> area of intact or scarified skin of the back of 3 New Zealand White rabbits. No reactions were recorded.

#### **Sensitization**

##### **Animal**

Twenty-five µL of the test material (62% Basic Red 76) was applied in concentrations of 2.5, 5, and 10% in a 7:3 v/v ethanol:water mixture.<sup>3,20</sup> Ten percent was the highest technically applicable concentration in the vehicle. 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 radiolabeled 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) was evaluated using 10 female Dunkin-Hartley guinea pigs.<sup>4</sup> Intradermal induction consisted of injections of the material solution (0.1% test material in water), Freund's Complete Adjuvant (FCA) diluted with an equal volume of water, and a 1:1 mixture of the material solution and FCA. 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 at a concentration of 25% w/v of the test material. Irritation was noted after administration of the intradermal injection in all animals, which was still present at the time of topical induction. Half of the test animals displayed erythema after the challenge phase that was resolved by 48 hours. The sensitization potential was considered to be equivocal.

### **OCULAR IRRITATION STUDIES**

#### **Animal**

An ocular irritation test was performed according to OECD TG 405.<sup>3,21</sup> The test material (62% Basic Red 76; 0.1 g) was instilled 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, and 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) in physiological saline was instilled into the conjunctival sac (0.1 mL) of one eye of each rabbit.<sup>4</sup> 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.

### **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 Red 76 is a direct 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 CIR Expert 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 Red 76 is a monoazo color that is reported to function 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 an arylazo-dehydrogenation reaction with an aromatic alcohol quickly results in an azo dye.

According to 2019 VCRP survey data, Basic Red 76 is reported to be used in 48 hair-coloring formulations and 2 nail polish and enamel 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 or aqueous formulation. Applications were performed in amounts of approximately 20 mg/cm<sup>2</sup>. The amount of the test material that was considered to be bioavailable by the direct dye cream and the aqueous solution was 1.96 ± 0.83 µg/cm<sup>2</sup> and 6.52 ± 3.58 µg/cm<sup>2</sup>, respectively. In a human study, 20 µL of 1 mM of the test substance (55.5% Basic Red 76) 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 diffused into the horny layer.

When an application of 200 µL of a hair setting lotion containing 0.1% test material (55.5% [<sup>14</sup>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<sup>2</sup> of skin. Other studies were performed in which a setting lotion and shampoo formulation containing 0.2 and 0.5% test material (55.5% [<sup>14</sup>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 (55.5% [ $^{14}\text{C}$ ]-labelled Basic Red 76), 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  $\mu\text{L}$  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% [ $^{14}\text{C}$ ]-labelled Basic Red 76) in physiological saline. 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  $\text{LD}_{50}$  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.

Decreases in organ weights and increases in the mean cell volume and hematocrit values were noted when Wistar MuRa Han 67 SPF rats were dosed via gavage 5 days a week for 12 weeks (test material, 55.5% Basic Red 76; 200 mg/kg bw). 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. The established NOAEL in a 90-day study involving Wistar rats dosed via gavage was 60 mg/kg/d (test material, 80.5% Basic Red 76).

No signs of developmental toxicity were observed in Wistar rats given 60 mg/kg bw/d on days 6 – 20 of gestation 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).

Mixed results were seen in Ames tests and mammalian gene mutation assays using Chinese hamster V79 cells with and without metabolic activation. Negative results were seen in a mammalian cell gene mutation assay using mouse lymphoma L5178Y cells. A chromosomal aberration assay using Chinese hamster V79 cells yielded negative results at up to 500 mg/mL; however, at the 1000 mg/mL concentration level, without metabolic activation, Basic Red 76 was clastogenic. An in vitro micronucleus test in V79 cells yielded positive results at concentrations as low as 212.5  $\mu\text{g/mL}$ , with and without metabolic activation; however, in part one of the same experiment, negative results were seen at concentrations as high as 300  $\mu\text{g/mL}$  with metabolic activation. Negative results were observed in in vivo micronucleus assays in mice at up to 5000 mg/kg.

No information regarding the carcinogenicity of Basic Red 76 was found. However, possible metabolites of Basic Red 76 (e.g., *o*-anisidine) have induced a number of multi-organ tumors according to several animal studies cited by NICNAS. (NICNAS, however, did not provide any specific evidence stating that Basic Red 76 is metabolized to *o*-anisidine.)

No irritation was reported when New Zealand White rabbits were dermally dosed with 500 mg or 0.5 g/in<sup>2</sup> of the test substance (62% Basic Red 76) under an occlusive patch. In an LLNA, Basic Red 76 was considered a non-sensitizer when 25  $\mu\text{L}$  of the test substance (62% Basic Red 76) was applied to mouse earlobes at a concentration of up to 10%. In a Magnusson-Kligman test, the sensitization potential of the test material (55.5% Basic Red 76) was evaluated using Dunkin-Hartley guinea pigs. Intradermal induction consisted of injections of the material solution (0.1% test material in water), Freund's Complete Adjuvant (FCA) diluted with an equal volume of water, and a 1:1 mixture of the material solution and FCA. 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 at a concentration of 25% w/v of the test material. Half of the test animals displayed erythema after the challenge phase that was resolved by 48 hours.

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.



The most recent comprehensive review of available epidemiology studies concluded that there is insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. A summary of the data is available in the CIR hair dye epidemiology Resource Document and can be found on the CIR website (<https://www.cir-safety.org/cir-findings>).

## **DISCUSSION**

Basic Red 76 is a direct dye that is reported to function in cosmetics as a hair-colorant and hair-conditioning agent. However, according to US FDA VCRP data, this ingredient is reported as being used in nail products. Use in nail products is not an approved use for this cosmetic ingredient, and therefore, the Panel is not reviewing the safety for such use.

The Panel found that the systemic toxicity, developmental/reproductive toxicity, genotoxicity, and irritation data in this report were sufficient. The Panel recognized the positive results of some genotoxicity studies, but considered them to be potentially misleading. Positive results were only obtained in in vitro studies, and at concentrations much higher than what would be used in cosmetics. All in vivo genotoxicity assays performed using Basic Red 76 yielded negative results, suggesting that the positive results seen in in vitro studies were not of concern. In addition, the Panel noted the carcinogenic potential of the aromatic amines formed by the metabolism of Basic Red 76. The concern regarding these metabolites was mitigated considering Basic Red 76 is poorly absorbed, is used in rinse-off products, and is used at very low concentrations. In addition, because use of this ingredient results in minimal dermal absorption, the actual exposure to these metabolized aromatic amines would be insignificant in light of cosmetic use conditions.

The Panel also considered the current recommendations for patch-testing, and noted that concerns over patch testing are ongoing. New methods for patch-testing using relevant protocols regarding application time and actual use conditions are continuously being developed. The Panel suggests that the patch-test instructions provided by manufacturers should be followed before hair dye use. In addition, hair dyes containing Basic Red 76, as coal tar hair dye products, are exempt from certain adulteration and color additive provisions of the Federal Food, Drug, and Cosmetic Act, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Expert Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures.

In considering hair dye epidemiology data, the Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other endpoints. Details of these studies can be found in the CIR hair dye epidemiology Resource Document.

## **CONCLUSION**

The CIR Expert Panel concluded that Basic Red 76 is safe for use as a hair dye ingredient in the present practices of use and concentration described in the safety assessment.

## TABLES

**Table 1. Physical and Chemical Properties<sup>3</sup>**

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 <sub>ow</sub>	-1.7834 ± 0.1131
Ultraviolet absorption λ maxima (nm)	235, 332, 503

**Table 2. Impurities/Components**

Basic Red 76 (% w/w)	62 <sup>3</sup>	80.5 <sup>3</sup>	89.1 <sup>3</sup>	55.5 (as chloride) <sup>4</sup>	> 77 (material used in the market) <sup>3</sup>
Water (% w/w)	5.1	4.1	3.1	NR	< 6
Monomethyl sulphate (% w/w)	11.8	15.9	11.4	NR	< 18
<i>o</i> -anisidine (%)	0.0005	0.0019	0.0011	NR	< 0.001
Chloromethane (% w/w)	0	0.3	0.1	NR	NR
Methyl acetate (% w/w)	0	0.1	NR	NR	NR
Methyl formate (% w/w)	0	0.4	NR	NR	NR
7-Hydroxy- <i>N,N,N</i> -trimethylnaphthalen-2-aminium chloride (%)	0	< 0.05	< 0.05	NR	NR
Methanol (% w/w)	0	0	0.7	NR	NR
Sulphated ash (% w/w)	0.4	0.3	0.1	NR	< 5
Chloride (% w/w)	1.6	2.7	4.4	NR	< 5
Sodium (%)	0.063	0.019	0.024	NR	NR
Calcium (%)	0.059	0	NR	NR	NR
Saccharose (% w/w)	25.8	0	NR	NR	NR
Sugar (undefined (%))	NR	NR	NR	16	NR
Volatile matter/water of crystallization (undefined (%))	NR	NR	NR	14	NR
Inorganic salts – chloride, sulfate, etc. (undefined (%))	NR	NR	NR	up to 100%	NR

NR = Not Reported

**Table 3. Frequency (2019) and Concentration of Use (2017) of Basic Red 76**

	# of Uses <sup>6</sup>	Conc of Use (%) <sup>7</sup>
<b>Totals*</b>	<b>48</b>	<b>0.057 - 0.35</b>
<b>FDA Product Categories</b>		
Hair Dyes and Colors	5	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)	24	NR
Nail Polish and Enamel	2	NR

NR = no reported use

**Table 4. Genotoxicity studies**

Test Article*	Concentration/Dose	Vehicle	Test System/Organism	Procedure	Results	Reference
<b>IN VITRO</b>						
80.5% Basic Red 76	Experiment 1: 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate Experiment 2: 33, 100, 333, 1000, 2500 and 5000 µg/plate	DMSO	<i>Salmonella typhimurium</i> (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	3,22
55.5% Basic Red 76	NR	NR	<i>S. typhimurium</i> (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.	4
80.5% Basic Red 76	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	3,23
55.5% Basic Red 76	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.	4
55.5% Basic Red 76	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.	4
80.5% Basic Red 76	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. Micronuclei induction was observed at concentrations associated with test item precipitation (212.5, 425, 850 µg/ml)	3,17

**Table 4. Genotoxicity studies**

Test Article*	Concentration/Dose	Vehicle	Test System/Organism	Procedure	Results	Reference
<b>IN VIVO</b>						
80.5% Basic Red 76	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. Bone marrow cells were collected 24 and 48 hours after a single administration. Toxicity was determined by measuring the ration of PCE and TE.	Non- clastogenic	3,18
55.5% Basic Red 76	5000 mg/kg bw	NR	CFW 1 mice (5/sex/group)	Micronucleus test; Mice were given doses of Basic Red 76 via gavage. This study was performed according to OECD 474. Duration of dosing was not provided. Animals were sacrificed at 24, 48, and 72 hours after treatment.	Non-clastogenic	4

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

\*further information about the composition of the tested materials can be found in Table 2

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