Safety Assessment of Basic Yellow 57 as Used in Cosmetics

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ABBREVIATIONS

CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
Dictionary	*International Cosmetic Ingredient Dictionary and Handbook*
DMSO	dimethyl sulfoxide
ECHA	European Chemicals Agency
FDA	Food and Drug Administration
HPLC	high performance liquid chromatography
IL-α	interleukin-1α
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect-level
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium salt
NCE	normochromatic erythrocytes
NOAEL	no-observable-adverse-effect-level
NOEL	no-observed-effect-level
NMR	nuclear magnetic resonance spectroscopy
OECD	Organization for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PCE	polychromatic erythrocytes
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
SCCNFP	Scientific Committee on Cosmetic and Non-Food Products
SCCS	Scientific Committee on Consumer Safety
TG	test guideline
US	United States
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Basic Yellow 57, 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 Yellow 57 is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

Basic Yellow 57 is reported to function as a hair colorant 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Ingredient Dictionary and Handbook* (wINCI; *Dictionary*).[1] This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated.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 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 Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Some chemical and toxicological data on Basic Yellow 57 included in this safety assessment were obtained from robust summaries of data submitted to the European Chemicals Agency (ECHA) by companies as part of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) chemical registration process.[2] Additionally, data were obtained from 2 opinions by the European Commission: 1 produced by the Scientific Committee on Cosmetic and Non-Food Products (SCCNFP), and the other produced by the Scientific Committee on Consumer Safety (SCCS).[3,4] These data summaries are available on the ECHA and European Commission's database, respectively, and when deemed appropriate, information from the summaries has been included in this report.

CHEMISTRY

Definition and Structure

Basic Yellow 57 (CAS No. 68391-31-1) is the monoazo hair color that conforms to the tautomers in Figure 1.[1, CIR Staff] Basic Yellow 57 is a direct dye that is used without mixing with an oxidizing agent (e.g. hydrogen peroxide).[4]

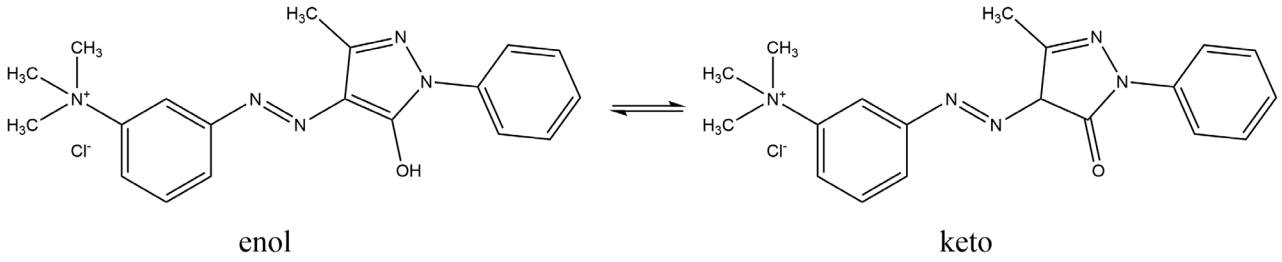

Figure 1. Basic Yellow 57 keto-enol tautomerism[CIR Staff]

Chemical Properties

Available chemical properties of Basic Yellow 57 are provided in Table 1. Basic Yellow 57 is soluble in water.[2] The SCCS has reported the measured log P_{ow} as 0.0632 (temperature not reported);[4] conversely, ECHA has reported the log P_{ow} to be 1.14 at 25 °C.[2]

Method of Manufacture

Basic Yellow 57 may be produced through the coupling of 3-amino-*N*,*N*,*N*-trimethylbenzenaminium chloride with 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one.[4,5] It is further reported in the SCCS opinion that the methylsulfate salt is not used as starting material; therefore no dimethylsulfate or monomethylsulfate is used or produced in the actual technical process.[4]

Composition/Impurities

The purities of 3 different batches of Basic Yellow 57 (the first being an actual market material containing sodium chloride as an extender and the last being a non-standardized batch without an extender) were 61%, 65% and, 78.7% (w/w) by nuclear magnetic resonance (NMR) spectroscopy and 98.7%, 99.0%, and 99.9% (area) by high performance liquid

chromatography (HPLC), all respectively.[4] The respective batches also contained water (5.5% - 9.2%), chloride (7.7% - 13.1%), sodium (1.05% - 6%), chloromethane (1.0%-1.6%, market material and non-standardized batches only), sulfate (0.3% - 0.8%), sulfated ash (3.6% - 24.9%), 5-methyl-2-phenyl-s,4-dihydro-3*H*-pyrazol-3-one (1100 ppm to below 10 ppm detection), and saccharose (24% in the second batch only). The market material also contained 10.6% methyl sulfate and 0.3% magnesium.

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 2021 VCRP survey data, Basic Yellow 57 is used in a total of 17 formulations. Of these reported uses, 5 are in hair dyes and colors, 4 are in coloring rinses, 3 are in coloring shampoos, 2 are in hair color sprays, and 3 are in "other" coloring hair products).[6; personal communication, N. Sadrieh, Sept 13, 2021] The results of the concentration of use survey conducted by the Council in 2021 indicate that Basic Yellow 57 is used at up to 0.43% in hair dyes and colors and up to 0.001% in coloring rinses and coloring shampoos.[7]

This ingredient is considered a coal tar hair dye for which regulations require caution statements and instructions regarding patch tests in order to be exempt from certain adulteration and color additive provisions of the 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.[8,9] 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 Yellow 57 is restricted to use only in non-oxidative hair dye products at a maximum concentration of 2.0% in ready for use preparations.[10] The SCCS in 2010 concluded that Basic Yellow 57 (not containing methyl sulfate) is safe for use in non-oxidative hair dye formulations with a maximum concentration of 2.0%, apart from possible sensitization potential.[4]

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

The percutaneous penetration/dermal absorption potential of Basic Yellow 57 (> 99% pure) through excised pig skin (400 µm thick) was determined from an aqueous solution and a representative direct dye formulation; both test materials contained 2% Basic Yellow 57.[3,4,11] Using Franz diffusion cells, each test material (10 mg/cm^2 of the aqueous solution and 10 µg/cm^2 of the standard formulation) was applied for 30 min to an exposure area of 2.54 cm^2; the resulting applications of Basic Yellow 57 were 197 and 180 µg/cm^2 with the aqueous and standard formulations, respectively. The receptor fluid (physiological saline and ethanol; 75:25) was analyzed at defined intervals for up to 48 h post application. The mean total recoveries of the test material in the aqueous solution and the dye formulation were 101% and 84.3%, respectively. For the aqueous solution and the dye formulation, most of the test material applied on the skin surface was removed with the washing (aqueous solution: 83.9% of applied dose after 30 min and a further 8.75% at the end of the exposure period; dye formulation: 76.7% of applied dose after 30 min and a further 2.51% at the end of the exposure period). Approximately 3.71% of the test material in the aqueous solution and 0.957% of the test material in the dye formulation was detected in the stratum corneum. A total of 3.0% of the applied dose (5.9 µg/cm^2) of the aqueous solution and 2.0% of the applied dose (3.5 µg/cm^2) from the dye formulation was found to have absorbed into the epidermis + dermis, and a total of 1.2% of the applied dose (2.4 µg/cm^2) of the aqueous solution and 0.5% of the applied dose (0.86 µg/cm^2) from the dye formulation was found to have penetrated into the receptor fluid during 48 h. Approximately 4.2% of the applied dose of the aqueous solution and 2.4% of the applied dose of the dye formulation were bioavailable. The penetration rate was 0.052 µg/cm^2/h for the aqueous solution and 0.018 µg/cm^2/h for the

standard. The SCCS noted the number of chambers used (6) was too few and an ethanolic receptor fluid was used; however, the experiment was acceptable and the value of 7.87 (mean + 2SD; 4.39 + 2 x 1.74) µg/cm^2 was used to calculate the margin of safety.

Animal

The potential for Basic Yellow 57 (purity not reported) to penetrate through the skin was studied in 3 Sprague Dawley rats (sex not specified).[3] The rats received 200 mg of a formulation containing 2.592 µCi [^{14}C]Basic Yellow 57 (0.1%) on clipped dorsal skin. The animals were collared to prevent licking of the application site. Excretion of radioactivity in urine and feces was measured for 24 h after application. The recoveries of radioactivity in urine and feces from 2 rats were very low, with less than 0.1% of the applied radioactivity in feces and less than 0.3% in urine. The third rat excreted more than 2.3% of the applied dose in the urine and 0.01% in the feces. The study was inconclusive. No further details provided.

Human

In a human dermal absorption study with 10 male subjects, applications of 20 µl of 1 mM Basic Yellow 57 (purity not reported) in 40% aqueous isopropanol were made on 5 separate skin areas (5.3 cm^2) of the inner forearm.[3] After 10 min and 6, 24, 48, and 72 h, the test sites of one treatment area 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. From the recovery rates, the amount of the test material that could possibly have penetrated the skin was estimated (details not provided). The test material diffused only to a minor degree into the horny layer, according to the corrected recovery rates. The researchers concluded that Basic Yellow 57 was not absorbed through the skin.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Acute dermal and oral studies summarized here are described in Table 2. In a dermal study in rats, the LD_{50} for Basic Yellow 57 was greater than 2000 mg/kg bw.[2] The LD_{50} for Basic Yellow 57 in an oral study in mice was 2350 mg/kg bw, while in studies using Sprague-Dawley rats, the LD_{50} was greater than 2000 mg/kg bw.[2-4] A study that tested Basic Yellow 57 at doses up to 4000 mg/kg bw in CFY rats found the LD_{50} to be between 1000 - 2000 mg/kg bw.[3]

Subchronic Toxicity Studies

Oral

In a 12-wk study, groups of 20 male and 20 female Wistar MuRa Han 67 SPF rats received 0 or 50 mg/kg bw of Basic Yellow 57 (purity not reported) in 10 ml/kg aqueous solution via gavage daily.[3] All rats were observed daily for clinical signs and mortality. Body weights and feed consumption were recorded weekly. Hematological analyses, clinical chemistry, and urinalysis were performed. At necropsy, organ weights were recorded and main organs were examined macroscopically and histologically. No adverse effects or mortalities were reported. The urine of treated animals was slightly colored. A small but significant reduction (less than 5%) in body weight gains in female rats was recorded for weeks 4 - 6 and at week 12. Hematological analyses showed an increase in mean cell volume and hematocrit of treated male rats. No treatment-related effects were observed in female rats. Clinical chemistry and urinalysis did not provide clear evidence of treatment-related effects. No differences were noted between control and treated animals during necropsy. The test material, tested at 50 mg/kg bw/d, was considered to be on the borderline for toxicity in this study.

The oral toxicity potential for Basic Yellow 57 (purity: 78.7% by NMR; 99.9 area% by HPLC) was studied in Wistar Crl:(WI)BR rats in a 90-d study.[4] Groups of 12 male and 12 female rats received 0, 100, 300, or 1000 mg/kg bw in Milli-U® water at a dose volume of 10 ml/kg bw via gavage, with additional groups of 5 males and 5 females receiving 0 or 1000 mg/kg bw for recovery investigations. The study was performed in accordance with Organization for Economic Cooperation and Development (OECD) test guideline (TG) 408. All rats were observed daily for clinical signs of toxicity. Body weights and feed consumption were recorded weekly. Ophthalmological examinations were made at the start and end of the study, and functional observation tests were performed at study end. The animals were then killed, and clinical pathology and macroscopy was performed. Organ weights and histopathology was performed on a selection of tissues (not detailed). The recovery groups were monitored for an additional 4 wk without treatment prior to being killed for routine pathology.

In the 1000 mg/kg dose group, 2 males and 4 females died prior to the end of the study. Two of the deaths were considered gavage errors; however, all 6 had enlarged spleen and extramedullary hemopoiesis that were treatment-related. Another 3 females in the 1000 mg/kg dose group died during the terminal blood sampling; the researchers determined that these deaths were related in part to the test material. The body weights and feed consumption of all treated animals were similar to controls. Hematology showed a dose-related effect on red blood cell turnover. Increased extramedullary hemopoiesis was observed in the 100 mg/kg dose group, which was more severe in the mid- and high-dose groups. Generalized bone marrow stimulation was indicated by increased reticulocyte counts at all doses, and increased number of platelets and sternal myelopoiesis in the 1000 mg/kg dose group. An increase in Heinz bodies was observed in the high dose

group and an increased methemoglobin formation was observed in all dose groups. The researchers considered the slightly higher percentage of methemoglobin in the low dose not an adverse effect. The increase in plasma bilirubin and potassium levels in the mid- and high-dose groups suggested that hemolysis occurred. The splenic terminal congestion in all dose groups indicate an increased extravascular sequestration of red blood cells by macrophages. At necropsy, enlargement and irregular surface of the spleen were observed with higher spleen weights. Red/orange/yellow staining of urine and various body parts, red contents of the urinary bladder, tinctorial change in the keratin of the stomach and tongue, and yellowish discoloration of various organs (including tongue, caecum, stomach mucosa, and mesenteric adipose tissue) in the 1000 mg/kg dose group were considered to be related to staining properties of the test material

At the end of the recovery period red blood cell counts were normal, but other hematological changes were noted (increased red cell distribution width, hemoglobin, hematocrit and mean corpuscular hemoglobin level). Increased spleen weights were noted in females, but without morphological correlates. Periacinar hepatocytic hypertrophy at 1000 mg/kg bw/d correlated with increased liver weight and liver enlargement. The higher alanine aminotransferase activity values at the high dose in males were considered to be due to the enlarged liver. In females, the decreased total protein level at mid and high dose, and increased prothrombin time in 1000 mg/kg dose group, suggested liver function effects. Based on the hematotoxic effects and spleen congestion, a lowest-observed-adverse-effect-level (LOAEL) for Basic Yellow 57 was determined to be 100 mg/kg bw/d, which can be corrected to 79 mg/kg bw when accounting for the purity of the test material.[4]

In another oral study, groups of 10 male and 10 female Sprague-Dawley CD rats received 0 or 20 mg/kg bw of Basic Yellow 57 (purity not reported) in 10 ml/kg aqueous solution via oral gavage daily for 13 wk.[3] The study was performed in the same manner as the study above, with the addition of ophthalmological examinations at the start and end of the study. No mortalities were reported. The body weight gain of treated animals was comparable to the control group. No treatment-related effects were observed. The no-observed-adverse-effect-level (NOAEL) was 20 mg/kg bw/d.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

In an oral teratogenicity study, a group of 23 pregnant Sprague-Dawley CD rats received 50 mg/kg Basic Yellow 57 (purity not reported) via gavage daily on days 6 to 15 of gestation.[3] A control group of 20 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 Yellow 57 was not considered teratogenic in rats at a dose of 50 mg/kg bw.

The teratogenic potential of Basic Yellow 57 (purity: 78.7% by NMR; 99.9 area% by HPLC) was studied in groups of female Wistar Crl:(WI)BR rats.[4] Groups of 24 females received 0, 100, 300, or 1000 mg/kg bw of the test material in Milli-U® water at a dose volume of 10 ml/kg bw via gavage. The rats received the test material once daily from day 6 to day 20 of gestation. The rats were checked daily for clinical signs of toxicity. Body weights and feed consumption were determined periodically during pregnancy. On gestation day 21, all females were killed and examined for external, thoracic, and abdominal macroscopic changes. The number of corpora lutea, implantation sites, resorptions, and living fetuses were counted in each litter. The weight of the gravid uterus, fetuses, and placenta, and the sex of the fetuses, were recorded. All fetuses were observed for macroscopic abnormalities. A portion of the fetuses of each litter were prepared and examined for skeletal or visceral abnormalities.

In the dams, 1 death was observed in the 1000 mg/kg dose group on gestation day 13, but the cause of death was unclear. A treatment-related effect could not be excluded. Body weights and body weight gains of the dams were comparable to the controls in the 100 and 300 mg/kg dose groups, but there was a statistically significant decrease of these parameters in the high dose group. Feed consumption was also decreased in the high dose group and in the 300 mg/kg dose group (from gestation day 12). Orange to red urine was noted in all animals in the 300 and 1000 mg/kg dose groups and in some of the 100 mg/kg dose group. An enlarged spleen was observed in 1 dam in the 300 mg/kg dose group and 5 dams in the 1000 mg/kg dose group. Crateriform retractions of the stomach were observed in 2 high dose dams. Abdominal fat and the forestomach appeared stained yellow in some of the high dose group, which were considered treatment-related. Pregnancy was not observed in 3 control, 2 low dose, 2 mid dose and 1 high dose dam. One dam in the 300 mg/kg dose group only showed implantation sites. Post-implantation losses increased in the 1000 mg/kg dose group, resulting in decreased fetal numbers and increased fetal deaths. Embryonic resorption was not affected. No treatment-related effects were seen at the other doses. The fetal sex ratio was not affected by treatment. In the low and mid dose groups, no treatment-related effects were seen. Maternal treatment with Basic Yellow 57 at dose levels up to 300 mg/kg bw/day did not elicit any teratogenic effects. At 1000 mg/kg, there was a high incidence of fetuses with major visceral abnormalities, including severe umbilical hernia and associated visceral changes, displacement of organs, and absence of the diaphragm. A number of the abnormal fetuses also exhibited malrotated hind limbs

and atypical skeletal ossification. Some ossification parameters showed slight retardation that could be explained by the reduction in mean fetal weight. The toxicological significance of the latter finding was unclear. Based on the results of this teratology study, the maternal no-observed-effect level (NOEL) was determined to be 100 mg/kg bw/d (79 mg/kg bw/d corrected for dye content) and the developmental NOAEL was determined to be 300 mg/kg bw/day (237 mg/kg bw/d corrected for dye content).[4]

GENOTOXICITY STUDIES

In vitro and in vivo genotoxicity studies on Basic Yellow 57 summarized here are detailed in Table 3. Basic Yellow 57 was not mutagenic in Ames tests at up to 5000 µg/plate,[2-4] nor in mouse lymphoma cells with and without metabolic activation at up to 1000 µg/ml.[4] However, some equivocal positive results were observed in Chinese hamster V79 cells, with a sporadic increase in mutant frequency at 1000 µg/ml without metabolic activation in 2 trials; with metabolic activation, inconsistent results were observed between the 2 trials regarding effect on mutant frequency.[3] Basic Yellow 57 was not clastogenic in Chinese hamster V70 cells in a micronucleus test at up to 2000 µg/ml, or a mammalian chromosomal aberration test at up to 1200 µg/ml.[3,4] No increase in unscheduled DNA synthesis was observed in rat hepatocytes exposed to up to 10,000 µg/ml Basic Yellow 57.[3] In vivo testing found that Basic Yellow 57 was not clastogenic or aneugenic in a mouse erythrocyte micronucleus test at up to 1000 mg/kg bw.

CARCINOGENICITY STUDIES

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

DERMAL IRRITATION AND SENSITIZATION STUDIES

Dermal irritation and sensitization studies on Basic Yellow 57 summarized here are detailed in Table 4. Basic Yellow 57 was predicted to be not irritating in EpiSkin™ irritation tests when tested neat.[2,12,13] No dermal irritation was observed in rats or rabbits tested with Basic Yellow 57 at up to 2000 mg/kg bw or 500 mg/kg bw, respectively.[2-4] Basic Yellow 57 was not sensitizing in a local lymph node assay (LLNA) study at up to 10%,[2,4] or in a guinea pig maximization test with a 0.1% intradermal induction, a 75% topical induction and a 25% challenge.[3]

OCULAR IRRITATION STUDIES

In Vitro

The ocular irritation potential of Basic Yellow 57 (99.3% pure by HPLC) was assessed using the MatTek EpiOcular™ 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, thiazolyl blue (MTT) assay in accordance with OECD TG 492.[2] The test material (50 mg) was applied to the cultured human-derived keratinocytes undiluted for approximately 6 h. Basic Yellow 57 was predicted to be irritating to the human eye.

Animal

In an ocular irritation study, 0.1 ml 0.5% Basic Yellow 57 (purity not reported) in physiological saline was instilled into the conjunctival sac of the left eye of 3 New Zealand White rabbits (sex not specified).[3] The right eye was treated with 0.1 ml of the vehicle and served as the control. Reactions were recorded at 30 and 60 min and 1 and 2 d after treatment. No effects were observed on the cornea or iris; however, discoloration of the conjunctivae was noted.

The ocular irritation potential of Basic Yellow 57 (99.3% pure by HPLC) was assessed in 1 male and 2 female New Zealand White rabbits in accordance with OECD TG 405.[2,4] The test material (0.1 g/animal) was instilled neat in the conjunctival sac of the left eye, while the other eye served as the untreated control. The eyes were then rinsed with tap water after 24 h. The eyes were observed for reactions 1, 24, 48, 72 h, and 7 and 10 d after instillation. Mild to moderate, early-onset, and transient ocular changes, such as reddening of the conjunctivae and sclerae, discharge, and chemosis, were observed. These effects were reversible and had disappeared by study end. No abnormal findings were observed in the cornea or iris in any animal, nor was corrosion observed. No clinical signs of toxicity were observed. Basic Yellow 57 was determined to be not irritating in this rabbit study.

MARGIN OF SAFETY

The SCCS calculated the margin of safety for a hair dye product that contained 2% Basic Yellow 57 to be 342.[4] This calculation is based on an adjusted LOAEL (/3; adjustment factor for the average LOAEL: NOAEL ratio [CIR Staff]) of 26 mg/kg bw/d (100 mg/kg bw, corrected to 79 mg/kg bw for 79% dye content) from a 90-d oral rat study and a systemic exposure dose (SED) of 0.076 mg/kg bw (skin area surface of 580 cm^2 x absorption through skin of 7.87 µg/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 (temporary or semi-permanent) dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes consist of preformed colors. Basic Yellow 57 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 Yellow 57 is reported to function as a hair colorant in cosmetic products, is a direct dye that is used without mixing with an oxidizing agent. According to 2021 VCRP survey data, Basic Yellow 57 is used in a total of 17 formulations. Of these reported uses, 5 are in hair dyes and colors, 4 are in coloring rinses, 3 are in coloring shampoos, 2 are in hair color sprays, and 3 are in "other" coloring hair products. The results of the concentration of use survey conducted by the Council in 2021 indicate that Basic Yellow 57 is used at up to 0.43% in hair dyes and colors and up to 0.001% in coloring rinses and coloring shampoos.

In a study using excised dermatomed pig skin, Basic Yellow 57 was bioavailable after 48 h. A dermal penetration study of Basic Yellow 57 (0.1% in formulation) in rats found low recoveries of the applied radioactivity in urine and feces; however, the study was determined to be inconclusive. In a human dermal absorption study, Basic Yellow 57 was not absorbed through the skin when 1 mM of the material in 40% aqueous isopropanol was tested.

In acute dermal study in rats, the LD_{50} for Basic Yellow 57 was greater than 2000 mg/kg bw. The LD_{50} for Basic Yellow 57 in an acute oral study in mice was 2350 mg/kg bw, while in studies using Sprague-Dawley rats, the LD_{50} was greater than 2000 mg/kg bw. A study that tested Basic Yellow 57 at doses up to 4000 mg/kg bw in CFY rats found the LD_{50} for Basic Yellow 57 between 1000 - 2000 mg/kg bw.

In a 12-wk oral study in rats that received 0 or 50 mg/kg bw Basic Yellow 57 by gavage, no mortalities were reported nor were adverse effects noted at necropsy. Small but significant reduction in body weight gains in female rats and an increase in mean cell volume and hematocrit in male rats indicated that the test material at 50 mg/kg bw/d was borderline for toxicity. In a 90-d feeding study in rats that received 0, 100, 300, or 1000 mg/kg Basic Yellow 57, hematology showed a dose-related effect on red blood cell turnover. Splenic terminal congestion was also noted in all dose groups. The LOAEL for Basic Yellow 57 was 100 mg/kg bw/d, which was corrected to 79 mg/kg bw to account for purity of the test material. No treatment-related effects were observed in a 13-wk oral study in rats tested with 0 or 20 mg/kg Basic Yellow 57; the NOAEL was 20 mg/kg bw/d.

In an oral teratogenicity study of Basic Yellow 57 in rats, the maternal NOEL was 100 mg/kg/d and the developmental NOAEL was 300 mg/kg/d, which was corrected to 237 mg/kg/d for dye content of the test material. Enlarged spleen were observed in dams that received 300 and 1000 mg/kg of the test material, and high incidences of fetuses with major visceral and skeletal abnormalities were observed at 1000 mg/kg. In another oral teratogenicity study in rats, Basic Yellow 57 did not produce adverse developmental effects when tested at 50 mg/kg bw/d.

Basic Yellow 57 was not mutagenic in Ames tests at up to 5000 µg/plate. Basic Yellow 57 was not mutagenic in mouse lymphoma cells with and without metabolic activation at up to 1000 µg/ml. However, some equivocal positive results were observed in Chinese hamster V79 cells, with a sporadic increase in mutant frequency at 1000 µg/ml without metabolic activation in 2 trials; with metabolic activation, inconsistent results were observed between the 2 trials regarding effect on mutant frequency. Basic Yellow 57 was not clastogenic in an in vitro micronucleus test using Chinese hamster V70 cells at up to 2000 µg/ml or a mammalian chromosomal aberration test at up to 1200 µg/ml. No increase in unscheduled DNA synthesis was observed in rat hepatocytes exposed to up to 10,000 µg/ml Basic Yellow 57. In vivo testing found that Basic Yellow 57 was not clastogenic or aneugenic in a mouse erythrocyte micronucleus test at up to 1000 mg/kg bw.

Basic Yellow 57 was predicted to be not irritating in EpiSkin™ irritation tests when tested neat. No dermal irritation was observed in rats or rabbits tested with Basic Yellow 57 at up to 2000 mg/kg bw or 500 mg/kg bw, respectively. Basic Yellow 57 was not sensitizing in an LLNA study at up to 10% or in a guinea pig maximization test with a 75% topical induction and a 25% challenge.

In an EpiOcular™ assay, Basic Yellow 57 was predicted to be irritating to the human eye. Ocular irritation was not observed in rabbit studies of Basic Yellow 57 when tested neat.

A margin of safety for a hair dye product that contained 2% Basic Yellow 57 was calculated to be 342. This calculation was based on an adjusted LOAEL (/3; adjustment factor for the average LOAEL: NOAEL ratio) of 26 mg/kg bw/d from a 90-d oral rat study and a SED of 0.076 mg/kg bw.

The Panel previously 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 carcinogenicity studies were found in the published literature, and unpublished data were not submitted.

DISCUSSION

Basic Yellow 57 is reported to function as a direct, non-oxidative hair dye in hair coloring products. 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. 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 lack of carcinogenicity studies for Basic Yellow 57. However, the Panel noted that Basic Yellow 57 absorbs slowly through the skin, is not genotoxic, and demonstrates minimal adverse systemic effects in animal toxicity studies. These findings, coupled with low use concentrations and short exposure time as a rinse-off product, helped mitigate concern over the absence of these data.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that Basic Yellow 57 is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Chemical properties for Basic Yellow 57

Property	Value	Reference
Physical Form	Orange-yellow fine powder	[4]
Molecular Weight (g/mol)	371.87	[4]
Density (g/cm^3) at 27.4 °C and 729 mm Hg	0.257 (mean pour); 0.334 (tapped)	[2]
Vapor Pressure (mmHg) at 25 °C	3.70×10^{-12}	[2]
Melting Point (°C)	163-169	[4]
(°C) at 728.8 mm Hg	148.5-156.3	[2]
Boiling Point (°C) at 728.8 mm Hg	250	
Water Solubility (g/l)		
at 28 °C	4.75	[2]
at 20 °C	> 100	[4]
log $P_{o/w}$ (temperature not reported)	0.0632	[4]
at 25 °C	1.140	[2]
UV-Vis Spectrum (200-800 nm) λ_{max} nm	384, 248	[4]

Table 2. Acute toxicity studies on Basic Yellow 57

Animals	No./Group	Vehicle	Dose/Protocol	LD_{50}/Results	Reference
			DERMAL		
Sprague-Dawley rats	5 male and 5 female/dose group	None	2000 mg/kg (purity not reported) in accordance with OECD TG 402; applied to shorn skin; test sites were occluded for 24 h and then rinsed with distilled water	> 2000 mg/kg bw; no skin reactions observed; no signs of toxicity or mortality; no abnormalities during gross pathological exam	[2]
			ORAL		
CF-1 mice	10 males/dose group	Olive oil	631, 1000, 2510, or 5010 mg/kg (purity not reported) via oral dosing; animals observed for 7 d; no further details	reported to be 2350 mg/kg bw (details not provided); clinical signs included increased respiratory rate and tremors; no further details	[3]
Sprague-Dawley rats	3 females/dose group	Distilled water	300 or 2000 mg/kg (purity not reported) via oral gavage in accordance with OECD TG 423	> 2000 mg/kg bw; no signs of toxicity or mortality for either dose group; no abnormalities during gross pathological exam	[2]
Sprague-Dawley rats	5 male and 5 female/dose group	Distilled water	2000 mg/kg (purity not reported) via oral dose; animals observed daily for 14 d for mortality and clinical abnormalities; body weights and macroscopic observations recorded but no histological exams were performed; no further details	> 2000 mg/kg bw; no mortalities; clinical signs included piloerection, hunched posture, abnormal gait, and increased salivation; body weights reported to be normal but no controls for comparison	[3,4]
CFY rats	2 male and 2 female/dose group	1% aqueous methylcellulose	0, 100, 1000, 2000, or 4000 mg/kg (purity not reported); observed daily for 14 d for mortality and clinical abnormalities; body weights and macroscopic observations recorded but no histological exams were performed; no further details	Between 1000-2000 mg/kg bw; one male and 2 females from the 2000 mg/kg dose group; all rat in 4000 mg/kg dose group died; clinical signs included piloerections and hunched posture, with lethargy and diarrhea at doses greater than 100 mg/kg; decreased respiratory rate, pallor of extremities, and ptosis at 1000 mg/kg; and increased salivation and diuresis at 2000 mg/kg	[3]

Table 3. Genotoxicity studies on Basic Yellow 57

Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
			IN VITRO		
3.16, 10, 31.6, 100, 316, or 1000 µg/plate; 99.9% pure (HPLC)	Dimethyl sulfoxide (DMSO)	*Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	Bacterial reverse mutation test in accordance with OECD TG 471; with and without S9 metabolic activation	Not mutagenic; no significant increase in the number of revertant colonies independent of metabolic activation	[2,4]
4, 8, 20, 40, 100, 200, 500, 1000, 2500, or 5000 µg/plate; purity not reported	DMSO	*S. typhimurium* strains TA98; TA100, TA1535, TA1537, TA1538	Bacterial reverse mutation test; appropriate positive and negative controls used; with and without metabolic activation	Not mutagenic; no dose-related or biologically relevant increase in revertant numbers observed in any strains, with or without metabolic activation	[3]
Test I with and without S9: 118.8, 237.5, 475.0, 712.5, or 950.0 µg/ml Test II without S9: 59.4, 118.8, 237.5, 475.0 or 950.0 µg/ml Test IIA without S9: 400, 500, 600, or 700 µg/ml 99.9% pure (HPLC)	Not reported	Mouse lymphoma cell line L5178Y	Mammalian cell gene mutation test at the thymidine kinase locus in accordance with OECD TG 476; with and without S9 metabolic activation; Test I cells were treated for 4 h and Tests II and IIA cells were treated for 24 h; each test was run using 2 parallel cultures each; positive controls were methyl methane sulfonate (without S9) and cyclophosphamide (with S9)	Not mutagenic; no relevant and reproducible increase of mutant frequency observed in Test I; in Test II, number of mutant colonies/10^6 cells exceeded the range of the historical control data at 237.5 µg/ml (culture II) and at 475 (both cultures), and there was a concentration-related increase except at the highest concentration, which was likely due to toxicity; in culture 2 of Test II, mutant frequency was increased 2.2 times over solvent control and induced mutant frequency was 163 x 10^{-6} at 475 µg/ml; a minor increase was observed in culture 1 and no concentration-related response was observed; the minor effects in Test II could not be repeated and were determined not biologically relevant; positive control results not reported	[4]
Test 1: 30, 100, 200, 300, or 1000 µg/ml with and without metabolic activation Test 2: 30, 100, 300, or 1000 µg/ml without metabolic activation and 20, 100, "250," 500, or 1000 µg/ml with metabolic activation 63.5% dye content	DMSO	Chinese hamster V79 cells	Mammalian cell gene mutation test at the HGPRT locus; with and without metabolic activation; cells were treated with the test material for 3 h with S9 and for 24 h without S9; appropriate positive and negative controls used	Some equivocal positive results, although accurate evaluation was confounded; a sporadic increase in mutant frequency at 1000 µg/ml without metabolic activation in both tests; with S9, a decrease in mutant frequency was observed in Test I at 200 µg/ml (concentration for which precipitate was noted) and greater, while a large increase in mutant frequency observed in Test 2 at 100 and 200 µg/ml and a decrease in the mutant frequency was observed at 500 and 1000 µg/ml (and precipitate was noted)	[3]
Test I without S9: 1000, 1500, or 2000 µg/ml Test I with S9: 1000, 1200, or 1400 µg/ml Test II without S9: 1000, 1500, or 2000 µg/ml 99.9% pure (HPLC)	Dulbecco's Modified Eagle Medium	Chinese hamster V79 cells	Micronucleus test in accordance with OECD TG 487; with and without S9 metabolic activation; Experiment I cells were treated for 4h and incubated for 18 h and Experiment II cells were treated for 24 h; positive controls were mitomycin C (without S9) and cyclophosphamide monohydrate (with S9)	Not clastogenic and/or aneugenic; no increase in the number of cells with micronuclei with or without metabolic activation in either experiment; micronucleus frequency of negative controls was partly below range of historical data, but positive controls yielded expected results and confirmed sensitivity of test	[4]
200-1200 µg/ml; > 99.2 area% HPLC	Deionized water	Chinese hamster V79 cells	Mammalian chromosomal aberration test in accordance with OECD TG 473; with and without metabolic activation; appropriate negative and positive controls used	Not clastogenic; no statistically and/or biologically significant relevant increase in number of aberrant cells observed at any dose with or without metabolic activation; no biologically relevant increase in the number of polyploid metaphases; controls yielded expected results	[3]
Test 1: 100, 330, 1000, 3330, or 10,000 µg/ml Test 2: 30, 100, 330, 1000, or 3330 µg/ml 63.5% dye content	DMSO	Rat hepatocytes	Unscheduled DNA synthesis in accordance with OECD TG 482; control information was not reported	No increase in unscheduled DNA synthesis observed, reduction in the incorporation of radioactivity noted at 3330 and 10,000 µg/ml; study was considered unsuitable for accurate evaluation by the SCCNFP	[3]

Table 3. Genotoxicity studies on Basic Yellow 57

Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
			IN VIVO		
0 or 1000 mg/kg bw in 20 ml/kg; purity not reported	0.9% NaCl solution	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 used	Not clastogenic and/or aneugenic; no significant variation in PCE/NCE ratio was observed, does not induce statistically significant increase in the frequency of PCE; controls yielded expected results	[3]

Table 4. Dermal irritation and sensitization studies on Basic Yellow 57

Concentration/Dose	Test Population	Procedure	Results	Reference
		IRRITATION		
		IN VITRO STUDIES		
Neat; purity not reported	Human skin tissue	EpiSkin™ skin irritation test; 15 min exposure	Not irritating; mean viability in sample 1 and sample 2 was 105% and 108%, respectively; calculated interleukin (IL)-1α release was 5 pg/ml for both samples; positive and negative controls yielded expected results	[2,12]
Neat	Human skin tissue	EpiSkin™ skin irritation test in accordance with OECD TG 439	Not irritating; mean viability was 115.4% (optical density) and 113.3 (HPLC/ultra-high-performance liquid chromatography performance spectrometry); positive and negative controls yielded expected results	[13]
		ANIMAL		
2000 mg/kg bw; 99.3% pure (HPLC)	5 male and 5 female Sprague-Dawley rats/dose group	In accordance with OECD TG 402 (acute dermal toxicity study summarized in Table 2); test sites shorn and occluded for 24 h with test material; rinsed with distilled water; observed for 14 d	Not irritating; erythema and edema scores were both 0	[2]
500 mg/animal in 0.1 ml purified water; 99.3% pure (HPLC)	1 male and 2 female New Zealand White rabbits	In accordance with OECD TG 404; semi-occlusive 4 cm^2 patches on clipped test sites for 4 h; scoring of reactions at 1, 24, 48, 72 h and 7 and 10 d post-patch removal	Not irritating; mean erythema/eschar and mean edema scores were both 0.00 in all animals	[2,4]
500 mg/animal in 0.5 ml distilled water; purity not reported	3 New Zealand White rabbits; sex not specified	Test material was applied to shorn intact or scarified skin for 24 h; occluded 2.54 cm^2 patch; reactions scored after 24 and 72 h	Not irritating	[3]
500 mg undiluted; purity not reported	3 male and 3 female New Zealand White rabbits	Test material was applied to shorn intact or scarified skin for 24 h; occluded 2.54 cm^2 patch; reactions scored upon removal of test material and daily for 14 d post-patch removal	Not irritating	[3]
		SENSITIZATION		
		ANIMAL		
0%, 2.5%, 5%, or 10% (w/v) in ethanol: water (7:3, v/v); 99.3% pure (HPLC)	Groups of 4 female CBA/CaOlaHsd mice	LLNA study in accordance with OECD TG 429	Not sensitizing; no clinical signs were observed; stimulation indices were 1.2, 1.5, and 1.5 for concentrations of 2.5%, 5%, and 10%, respectively	[2,4]
0.1% w/v intradermal induction; 75% w/v topical induction; challenge 25% and 5% (w/v); in distilled water; purity not reported	10 female Dunkin-Hartley guinea pigs	Guinea pig maximization test in accordance with OECD TG 406; intradermal induction included Freund's complete adjuvant followed 1 wk later with topical induction; 2 wk after induction, animals challenged with 25% test material followed 1 wk later with 5%	Not sensitizing; intradermal induction caused an irritation response that continued to time of topical induction; erythema was observed in 7/10 animals after 1st challenge; subsequent 5% challenge applied to determine if erythema was an irritation or sensitization response; 2/10 animals had erythema to 2nd challenge that resolved within 48 h	[3]

REFERENCES

1. Nikitakis J, Kowcz A. Web-Based International Cosmetic Ingredient Dictionary and Handbook. http://webdictionary.personalcarecouncil.org/jsp/Home.jsp. Washington, DC: Personal Care Products Council. Last Updated 2021. Accessed 03/05/2021.

2. European Chemicals Agency (ECHA). 3-[(4,5-dihydro-3-methyl-5-oxo-1-phenyl-1H-pyrazol-4-yl)azo]-N,N,N-trimethylanilinium chloride. https://echa.europa.eu/registration-dossier/-/registered-dossier/17312 2021. Accessed: 03/24/2021.

3. Scientific Committee on Cosmetic and Non-Food Products (SCCNFP). Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers Concerning Basic Yellow 57 (Colipa No. C10). 2003. SCCNFP/0679/03. https://ec.europa.eu/health/ph_risk/committees/sccp/documents/out217_en.pdf. Accessed 03/30/2021.

4. Scientific Committee on Consumer Safety (SCCS). Opinion on Basic Yellow 57 (COLIPA no. C10). 2010. SCCS/1231/09. https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_020.pdf. Accessed 04/05/2021.

5. World Dye Variety. Basic Yellow 57. http://www.worlddyevariety.com/basic-dyes/basic-yellow-57.html. Last Updated Accessed 05/14/2021.

6. US Food and Drug Administration (FDA) Center for Food Safety & Applied Nutrition (CFSAN). 2021. Voluntary Cosmetic Registration Program (VCRP) - Frequency of Use of Cosmetic Ingredients. College Park, MD Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2021; received January 21, 2021.

7. Personal Care Products Council. 2021. Concentration of Use by FDA Product Category: Basic Yellow 57. Unpublished data submitted by the Personal Care Products Council on January 6, 2021.

8. Thyssen JP, Sosted H, Uter W, et al. Self-testing for contact sensitization to hair dyes - scientific considerations and clinical concerns of an industry-led screening programme. *Contact Dermatitis*. 2012;66(6):300.

9. Goossens A. Self-testing for contact sensitization to hair dyes. *Contact Dermatitis*. 2012;66(6):299.

10. European Commission. Cosing database; following Cosmetic Regulation (EC) No. 1223/2009 http://ec.europa.eu/growth/tools-databases/cosing/. Last Updated 2020. Accessed 4/9/2021.

11. Steiling W. The bioavailability of cationic azo hair dyes: Application of a new *in vitro* method. *Altern Lab Anim*. 2004;32(Suppl 1B):689-692.

12. Scientific Committee on Consumer Safety (SCCS). Memorandum (addendum) on the *in vitro* test EPISKIN™ for skin irritation testing. 2010. SCCS/1392/10. https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_s_003.pdf. Accessed 05/07/2021.

13. Alepee N, Hibatallah J, Klaric M, Mewes KR, Pfannenbecker U, P. M. Assessment of cosmetic ingredients in the in vitro reconstructed human epidermis test method EpiSkin™ using HPLC/UPLC-spectrophotometry in the MTT-reduction assay. *Toxicol In Vitro*. 2016;33:105-117.