Safety Assessment of Tetrabromophenol Blue as Used in Cosmetics

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All interested persons are provided 60 days from the above release date (i.e., by February 11, 2025) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume, M.B.A. This safety assessment was prepared by Christina Burnett, M.S., Senior Scientific Analyst/Writer, CIR.

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ABBREVIATIONS

AUC	area under the curve
C_{max}	maximum observed concentration
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook*
DMSO	dimethyl sulfoxide
EPA	Environmental Protection Agency
FD&C	Food, Drug, and Cosmetic
FDA	Food and Drug Administration
HPLC	high-performance liquid chromatography
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LLNA	local lymph node assay
MoCRA	Modernization of Cosmetics Regulation Act
MOE	margin of exposure
MOS	margin of safety
NOAEL	no-observable-adverse-effect-level
NR	not reported
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
RLD	Registration and Listing Data
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dose
SI	stimulation index
$t_{1/2}$	half-life
TG	test guideline
US	United States
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of Tetrabromophenol Blue, which is reported to function as a hair colorant in cosmetic products. The Panel reviewed the available data to determine the safety of this ingredient. The Panel concluded that Tetrabromophenol Blue is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This assessment reviews the safety of Tetrabromophenol Blue as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), this ingredient is reported to function as a hair colorant in cosmetic products.[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 extensive search of the world's literature; a search was last conducted October 2024. 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.

Much of the data included in this safety assessment was found in opinions prepared by the European Commission's Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) and Scientific Committee on Consumer Safety (SCCS).[2-5] Please note that the SCCNFP and SCCS opinions provide summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when the SCCNFP and SCCS are cited.

CHEMISTRY

Definition and Structure

According to the *Dictionary*, Tetrabromophenol Blue (CAS No. 4430-25-5) is the organic compound that conforms to the structure in Figure 1.[1] While the ingredient name may give the erroneous impression that there are just 4 bromine substituents, this ingredient most commonly contains 8 bromine substituents (as much as 90% of Tetrabromophenol Blue is octabromo-substituted), with considerably fewer instances with only 7 (and fewer still instances of 6). The full systematic name of this chemical, 4,5,6,7-tetrabromo-3,3-bis(3,5-dibromo-4-hydroxyphenyl)-3H-benzo[c][1,2]oxathiole 1,1-dioxide, is simply too long for product labels, but does denote all 8 substitutions.

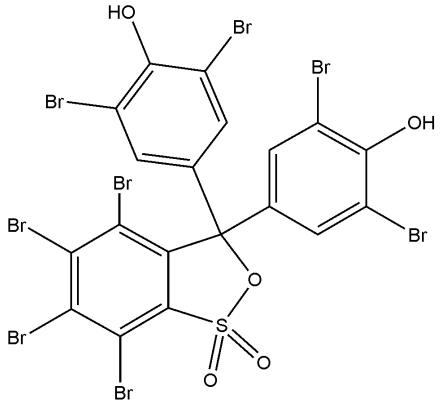

Figure 1. Tetrabromophenol Blue

Tetrabromophenol Blue is used in oxidative and non-oxidative hair dye formulations.[4,3,5] It is a polybrominated phenolsulfonphthalein.[6]

Chemical Properties

Chemical properties of Tetrabromophenol Blue are summarized in Table 1. Tetrabromophenol Blue is a yellowish-grey powder with a molecular weight of 985.59 g/mol.[5,3,4,2] UV absorption (λ_{max}) is reported at 224, 299, and 610 nm.[5,3,4] The calculated log P_{ow} is 5.98 ± 0.20.[2,3,5,4]

Method of Manufacture

Method of manufacture data were not found in the published literature, and unpublished data were not submitted.

Composition/Impurities

Tetrabromophenol Blue is a mixture of octa-, hepta-, and hexa-bromo phenolsulfonphthaleins.[3,5,4,2] A supplier reported to the SCCS that the purity of Tetrabromophenol Blue, when manufacturing processes control for hexabromo-homologue (Figure 1) and one type of heptabromo-homologue (Figure 2; molecular weight of 906.65 g/mol), is > 98.5%.[3]

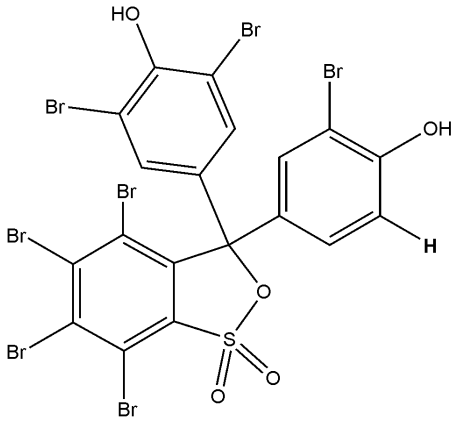

Figure 2. The heptabromo-homologue, heptabromophenolsulfonphthalein

According to this supplier, Tetrabromophenol Blue may comprise as much as 16% of the heptabromo-homologue. Heavy metal content was reported to be < 1 ppm for lead, mercury, and arsenic each, respectively. n-Propanol, a solvent residue, was reported to be 4.86% w/w. The impurities acetylated octa-homologue, acetylated hexa-homologue, benzylated octa-homologue, and benzylated hexa-homologue were present at < 0.09%.

The above was considered by the SCCS, in their 2019 dossier, to be the then current composition and impurities of Tetrabromophenol Blue in use on the market ("market quality"). This composition, when contrasted to toxicological study test material compositions, represents a significantly higher proportion of those 2 chemicals with structures shown in Figures 1 and 2, than other Tetrabromophenol Blue homologues.

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. Data included herein were obtained from the FDA and in response to a survey of maximum use concentrations conducted by the Personal Care Products Council (Council), and it is these values that define the present practices of use and concentration. Frequencies of use obtained from the FDA include data from the Voluntary Cosmetic Registration Program (VCRP) database as well as Registration and Listing Data (RLD). As a result of the Modernization of Cosmetics Regulation Act (MoCRA) of 2022, the VCRP was terminated in 2023, and as of 2024, manufacturers and processors have been mandated to register and list their products (and ingredients therein) with the FDA (i.e., RLD). However, because there are numerous differences in the ways the data for the VCRP and the RLD were collected and processed, and because reporting frequency of use is now mandatory (as opposed to the past practice of voluntary reporting), at this time it is not appropriate to contrast data from the VCRP and RLD to determine a trend in frequency of use. Although the VCRP program is now defunct, trends in frequency of use from the RLD alone are not yet possible in that a baseline is currently not available.

According to RLD that CIR received in 2024, Tetrabromophenol Blue is reported to be used in 40 hair coloring preparations (Table 2).[7] The 2023 VCRP survey data reported Tetrabromophenol Blue to be used in 2 formulations, 1 hair lightener with color and 1 hair bleach.[8] The results of the concentration of use survey conducted by the Council indicate Tetrabromophenol Blue is used at 0.0025% in hair dyes and colors.[9]

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 (FD&C) 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.[10,11] Hair dye products marketed and sold in the US, though, must follow the labeling requirements established by the FD&C Act.

Some products containing Tetrabromophenol Blue may be marketed for use with airbrush delivery systems. With the advent of MoCRA and the current product categories outlined by the FDA, it is now mandatory that cosmetic products used in airbrush delivery systems be reported as such in the RLD. In other words, a reliable source of frequency of use data regarding the use of cosmetic ingredients in conjunction with airbrush delivery systems is now available. Additionally, the Council currently surveys the cosmetic industry for maximum reported use concentrations of ingredients in products which may be used in conjunction with an airbrush delivery system; thus, this type of data may also be available when submitted. However, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety. Without information regarding the consumer habits and practices data or product particle size data (or other relevant particle data, e.g., diameter) related to this use technology, the data profile is incomplete, and the Panel is not able to determine safety for use in airbrush formulations. Accordingly, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

In the European Union, Tetrabromophenol Blue is categorized in Annex III, the list of substances which cosmetic products must not contain except subject to the restrictions laid down.[12] For this ingredient, the regulation states that the maximum concentration applied to the hair must not exceed 0.2% in both oxidative and non-oxidative hair dye products. In 2019, the SCCS concluded that Tetrabromophenol Blue is safe in oxidative and non-oxidative hair coloring products at a final on-head concentration of up to 0.2%.[3]

Non-Cosmetic

Tetrabromophenol Blue has been researched for use in bioassays for detecting proteinuria.[13-19] Other non-cosmetic uses reported include industrial dye, a laboratory indicator, and a biological stain.[6]

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

The cutaneous absorption of an oxidative formulation containing 5% Tetrabromophenol Blue (96.7 - 98.8% pure) was determined using full thickness pig skin (1000 µm thick) mounted on diffusion cells.[3,5,4,2] This study was performed in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 428. A dose of 400 mg of the formulation was applied on skin samples (n = 6; 1.67% Tetrabromophenol Blue on 4 cm^2) for 30 min before it was rinsed off with water and shampoo. After 72 h, the amount of the test material was determined in the receptor fluid, in the skin (epidermis and upper dermis separated), and in the rinsing solution. The content of Tetrabromophenol Blue in all fractions in the receptor fluid was below the limit of quantification (56 ng/cm^2 per fraction). Considering the limit of quantification as the upper limit, the amount of Tetrabromophenol Blue in the receptor fluid was < 0.339 µg/cm^2 (< 0.02% of the applied dose). Thus, the amount of < 0.339 µg/cm^2 was considered to have passed the skin barrier during the 72-h period (worst case assumption included the amount of test material in the upper dermis, yielding a maximum dermal penetration rate of 0.379 µg/cm^2/72 h). The concentrations of Tetrabromophenol Blue detected in the separated skin layers were 0.901 ± 0.116 µg/cm^2 (0.054 ± 0.007%) in the epidermis, and 0.04 ± 0.013 µg/cm^2 (0.002 ± 0.001%) in the upper dermis. A total recovery of 95.1% was calculated, including the amount of test material in the rinsing solution (1584 µg/cm^2, or 95%). The SCCS in 2012 determined this study was inadequate due to methodological issues, including lack of exact composition of the formulation, use of full thickness skin, use of an infinite dose application of the formulation, and incorrect absorption calculations.

In another cutaneous absorption study, a formulation containing 0.2% Tetrabromophenol Blue was applied to split thickness pig skin (1120 µm thick) mounted on permeation chambers (5 chambers with formulation and 1 chamber was a control).[4,5,3] This study was performed in accordance with OECD TG 428. The purity of the Tetrabromophenol Blue used in the study was characterized using high-performance liquid chromatography (HPLC) to be the following: 38.2 area% at 210 nm, 45.1 area% at 254 nm, 47.1 area% at 615 nm and 96.7 area% for all brominated homologues at 210 nm. After application of 100 mg/cm^2 formulation for 60 min, the skin samples were rinsed with water and shampoo. The recovered Tetrabromophenol Blue was found mainly in the rinsings (92.42 ± 1.72%, or 184.83 ± µg/cm^2). Small amounts of Tetrabromophenol Blue were found in the upper skin (1.10 ± 0.45%, or 2.20 ± 0.89 µg/cm^2). Tetrabromophenol Blue was not detectable in the physiological receptor fluid fractions collected within 72 h and in the separated lower skin compartments after 72 h. Taking into account the estimates from the limits of detection, 2.71 ± 0.89 µg/cm^2 of Tetrabromophenol Blue was considered as biologically available. The SCCS commented that only 5 chambers were used in this study and the dose of the dyes was too high.

The percutaneous absorption potential of 0.2% (w/w) ^{14}C-Tetrabromophenol Blue in a non-oxidative hair dye formulation was analyzed using frozen dermatomed human skin (380 - 400 µm thick).[4,3] The study was performed in accordance with OECD TG 428. The purity of Tetrabromophenol Blue was 96.3% for non-radiolabeled material and 99.4% for radiolabeled material. For 30 min, 20 mg/cm^2 of the test material was applied occluded to skin samples (12 replicates

from 5 donors) mounted into flow-through diffusion cells (exposed surface area: 0.64 cm^2). The receptor fluid was pumped through the receptor chambers at 1.5 ± 0.15 ml/h. The absorption of Tetrabromophenol Blue was evaluated by collecting receptor fluid (minimum essential medium) in 30 min fractions from 0 to 1 h post-dose, in hourly fractions from 1 to 6 h post-dose, and in 2-hourly fractions from 6 to 72 h post-dose. At 30 min post-dose, the parafilm occluding the chambers was removed and retained for analysis. The skin was washed with water and sodium dodecyl sulfate solution (2% w/v), and then dried with tissue paper swabs. At 72 h post-dose, the skin surface was washed and dried in the same manner. The underside of the skin was rinsed with receptor fluid, and then removed from the flow-through cells and dried. The skin was divided into exposed and unexposed samples. The stratum corneum was removed by tape stripping. The exposed epidermis was then heat-separated from the dermis, and skin compartments were extracted separately. The radioactivity was quantified by liquid scintillation counting.

The validity of the test was confirmed with a total recovery within the range of 100 ± 10% of the applied dose for all skin samples. The majority of the applied dose of Tetrabromophenol Blue was rinsed off from the skin surface at 30 min post-application, representing 65.77%. At 72 h, 9.54 ± 3.07 µg/cm^2 (22.43 ± 7.22%) of Tetrabromophenol Blue was recovered from the stratum corneum. From the dermis, 0.02 ± 0.02 µg/cm^2 (0.06 ± 0.05%) was recovered. From the epidermis, 1.62 ± 1.96 µg/cm^2 (3.82 ± 4.57%) was recovered. A maximum amount of 0.03 ± 0.01 µg/cm^2 (0.07 ± 0.02%) Tetrabromophenol Blue passed through the skin and was recovered in the receptor fluid during the 72-h exposure. A total amount of 0.05 ± 0.02 µg/cm^2 Tetrabromophenol Blue was considered to be bioavailable.[4,3]

The same study group performed a percutaneous absorption study (in the same manner described above) using the same batch of Tetrabromophenol Blue at 0.2% in an oxidative hair dye formulation.[4,3] The test was validated with total recovery being within the range of 100 ± 10% of the applied dose for all skin samples. The majority of the applied dose of Tetrabromophenol Blue was rinsed off from the skin surface at 30 min post application, representing 95.31%. At 72 h, 0.34 ± 0.18 µg/cm^2 (0.73 ± 0.38%) of Tetrabromophenol Blue was recovered from the stratum corneum. Less than 0.01% was recovered from the dermis. From the epidermis, 0.05 ± 0.06 µg/cm^2 (0.10 ± 0.13%) was recovered. The absorbed dose of Tetrabromophenol Blue, which was also considered the amount that was bioavailable, was determined to be 0.02 ± 0.02 µg/cm^2 (0.05 ± 0.04%).

Absorption, Distribution, Metabolism, and Excretion

Animal

Dermal

In toxicokinetic studies, groups of female Wister CRL: WI BR (outbreed) SPF rats received 9 mg/kg bw (0.09 mg/cm^2 skin, 9 mg/ml) ^{14}C-Tetrabromophenol Blue in water/acetone (1:1).[4,5,3] The purity of the non-labelled chemical was 97.5% and the radiochemical purity was 88.8%. The study was performed in accordance with OECD TG 417 and 427. In the mass balance groups (n per group = 4), the rats were housed in metabolism cages in order to obtain a total ^{14}C-radioactivity material balance. After dosing, urine and feces were collected over time intervals of 0 - 8 h, 8 - 24 h, 24 - 48 h, 48 - 72 h, and 72 - 96 h. The animals were killed after 96 h and several tissues and organs were collected. Total radioactivity in urine, feces, tissues, and organs was determined. For metabolic studies, urine and feces were pooled per group (n per group = 6), and the metabolite profile of the pooled samples was obtained by HPLC and liquid chromatography – tandem mass spectrometry (LC-MS/MS). In the toxicokinetic groups, blood was sampled alternately from several rats per time point at 15 and 30 min, and at 1, 2, 4, 8, 24, and 48 h. Total radioactivity of Tetrabromophenol Blue equivalent concentrations was determined.

No mortality was observed. After dermal dosing in the mass balance and toxicokinetics groups, chromodacryorrhea from the nose and eye was observed. This effect was not related to grooming as the rats had neck collars. After dermal application, the mean cumulative recovery of radioactivity was 0.013 ± 0.007% of the dose for the urine and 0.838 ± 0.248% of the applied dose for the feces. Mean residual radioactivity in the carcass and tissues (without skin) was 0.314%. The recovery from the treated skin was 0.369 ± 0.151%. Less than 0.05% of the total radioactivity was recovered in the cage wash. The mean mass balance was 97.332 ± 2.521%. Excretion via the feces and urine was low (0.8 and 0.01%, respectively), indicating poor dermal absorption. Further details on the recovery of the radioactivity were not provided. A toxicokinetic evaluation could not be performed. It was concluded that dermal absorption of 0.9% aqueous ^{14}C-Tetrabromophenol Blue was 1.2% of the applied dose.[4,5,3]

Oral

The same animal study groups of the dermal study described above were used to analyze the toxicokinetics of the same batch of ^{14}C-Tetrabromophenol Blue via gavage.[4,5,3] The test material was administered at doses of 10 or 100 mg/kg bw in 5.3% w/w polyglycol 600, 4.2% w/w of trade named alkyl polyglucoside containing 50% aqueous decyl glucoside, and 90.5% ultrapure water. Mass balance groups were comprised of 4 female rats per dose, while toxicokinetic groups were comprised of 6 female rats per dose. In the toxicokinetic groups, blood was sampled alternately from several rats per time point at 15 and 30 min, and at 1, 2, 4, 8, 24, and 48 h. The analytical methods were the same as those described above.

One rat of the low-dose group died on day 2 of the study, most likely as a result of mis-dosing. No clinical signs of toxicity were observed. After oral dosing, the mean cumulative recovery of ^{14}C-Tetrabromophenol Blue in the urine after 96 h was 0.031 ± 0.004% (low-dose) and 0.03 ± 0.001% (high-dose) and in feces was 107.1 ± 5.06% (low-dose) and 119.5 ±

6.618% (high-dose). Mean residual radioactivity in the carcass, tissues, and blood was 0.244% (low-dose) and 0.353% (high-dose). Less than 0.02% of the total radioactivity was recovered in the cage wash. The mean mass balance was 107.40 ± 5.03% (low-dose) and 119.9 ± 6.63% (high-dose). The main route of excretion of Tetrabromophenol Blue and its metabolites was through the feces, with 107 - 119% of the administered dose recovered in the feces. Analysis of pooled samples from excreta did not yield sufficient information on the metabolites to propose a definite chemical structure. Excretion in urine was 0.03 - 0.1% of the administered dose. Oral toxicokinetics was linear with maximum observed concentration (C_{max}) values of 0.431 mg/kg bw (low-dose) and 7.32 mg/kg bw (high-dose). The area under the curve ($AUC_{0-\infty}$) values were 4.58 and 111.0 $mg_{eq}h/kg$ for the low- and high-dose groups respectively. The dose-normalized AUC values were in the same order of magnitude, i.e. 0.450 and 1.070 $mg_{eq}h/kg$, respectively. Apparent terminal half-lives ($t_{1/2}$) of ^{14}C-Tetrabromophenol Blue were also similar in both dose groups with 19 and 15 h, respectively. It was concluded that after oral administration, ^{14}C-Tetrabromophenol Blue was moderately absorbed, readily distributed into all organs, and excreted mainly via the feces. The oral absorption of ^{14}C-Tetrabromophenol Blue was 30% (10 mg/kg) and 29% (100 mg/kg) of the applied dose.[4,5,3]

Parenteral

The same animal study groups of the dermal and oral studies described above also were used to analyze the same batch of ^{14}C-Tetrabromophenol Blue following intravenous administration.[4,5,3] The rats received 5 ml/kg of the test material in 0.05 M phosphate buffer (pH 7.6). Mass balance groups were comprised of 4 female rats per dose, while toxicokinetic groups were comprised of 6 female rats per dose. In the toxicokinetic groups, blood was sampled alternately from several rats per time point at 15 and 30 min, and at 1, 2, 4, 8, 24, and 48 h. The analytical methods were the same as those described previously. No mortalities or clinical signs of toxicity were reported. The mean percent recovery of radioactivity after 96 h was 0.102 ± 0.013% in urine and 112.76 ± 14.30% in feces. The mean residual radioactivity in the carcass and tissues was 5.89% of the dose. Less than 0.05% of the total radioactivity was recovered in the cage wash. The mean mass balance was 113.49 ± 14.32%. Excretion in urine was 0.03 - 0.1% of the administered dose. After intravenous administration, $t_{1/2}$ was 23.04 h.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Acute toxicity studies were not found in the published literature, and unpublished data were not submitted.

Short-Term Toxicity Studies

Oral

In a 28-d study, groups of 5 male and 5 female SPF-bred Wistar rats received Tetrabromophenol Blue (96.7 - 98.8% pure) dissolved in water containing 5.3% polyglycol 600 and 4.2% of a 50% aqueous decyl glucoside solution via gavage.[2,5] The dose levels were 0, 3, 10, and 100 mg/kg bw/d. This study was performed in accordance with OECD TG 407. Clinical observations for toxicity were made once daily, and during week 4, functional observations including a motor activity test were performed. Body weights and feed consumption were measured weekly. At study end, clinical biochemistry, macroscopic, microscopic, and histopathology examinations were performed, and organ weights were measured. Organs and tissues (details not provided) were analyzed from all the rats in the 100 mg/kg bw/d dose group and the controls.

No treatment-related deaths occurred. Blue discoloration of the feces and other body parts was observed in the 10 and 100 mg/kg bw/d dose groups. No relevant substance-related clinical findings were noted. No treatment-related effects were noted in functional observations, body weight gain, or feed consumption. Rats in the 100 mg/kg bw/d dose group had an increase in white blood cell counts in the males and increases in cholesterol and glucose in the females. At 10 mg/kg bw/d, only 1 male had a high glucose value. Discoloration of the cecum was observed in high dose animals; this effect was due to the staining properties of the test material. Evaluation of substance-related changes in absolute and relative body wights could not be performed due to missing values in the male controls. The no-observable-adverse-effect level (NOAEL) was determined to be 3 mg/kg bw/d.[2,5]

Subchronic Toxicity Studies

Oral

In a study of the same test material using the same dose levels as the 28-d study described above, groups of 10 male and 10 female SPF-bred Wistar rats received Tetrabromophenol Blue daily via gavage for 91 d (males) or 92 d (females).[2,5,4,3] This study was performed in accordance with OECD TG 408. In addition to the previously described methodology, ophthalmoscopy was performed pre-dosing and at week 13 of the study. Lungs, livers, and kidneys of all dose groups were examined, while other organs and tissues (details not provided) were analyzed from the highest dose group and controls.

No treatment-related deaths occurred. Blue discoloration of the feces and the fur was observed in all dose groups. Alopecia, chromodacryorrhea, and other skin problems were also common in all dose groups, but these were considered to be within the normal range. However, chromodacryorrhea increased in a dose-related manner in females. By the end of the dosing period, these effects were more pronounced, both in numbers affected and with increasing severity of the response in the mid- and high-dose groups. Three females that had chromodacryorrhea (1 mid- and 2 high-dose) also exhibited behavioral effects (hunching, piloerection, and clonic spasms). No treatment-related effects were noted in functional

observations, body weight gain, or feed consumption. During ophthalmoscopy, multifocal corneal opacities were observed in 1 male of the 10 mg/kg bw/d group and in 4 males in the 100 mg/kg bw/d group; this effect may have been due to corrosive properties of the test substance and direct eye contact with the fur. Statistically significant, but not dose-related, differences in hemoglobin and hematocrit values between the dose groups were observed pre-dosing and at the end of the study. These effects were not considered toxicologically relevant, but in males, changes in platelet values at 100 mg/kg bw/d and in erythrocyte counts, which were statistically significant at 10 and 100 mg/kg bw/d, indicated a hematotoxic potential of the test material. At 100 mg/kg bw/d, changes in urea (males) and cholesterol (female) values were found. Discoloration of the gastrointestinal tract was observed, which was due to the staining properties of the test material. The NOAEL was determined to be 10 mg/kg bw/d by the study authors, but the SCCNFP determined the NOAEL should be 3 mg/kg bw/d due to the ophthalmological and hematological findings.

The SCCS further noted that chromodacryorrhea was not considered toxicologically significant; however, these could have been cholinergic effects that are associated with non-specific responses to stress.[3,5,4] Behavioral changes in 3 females that had chromodacryorrhea support this. When taken in conjunction with the higher incidence of corneal opacities in males, the ophthalmic effects observed were likely systemic effects than produced by direct contact. In additional SCCS notes, the statistically significant reduced platelet and urea values in high dose males and increased cholesterol values in high dose females were not considered to be toxicologically significant as these were within the normal variation of rats of their age and strain.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

The developmental toxicity potential of Tetrabromophenol Blue (96.7 - 98.8% pure) was assessed in groups of 24 female SPF-bred Wistar rats treated on gestation days 6 - 20 in a study performed in accordance with OECD TG 414.[3,5,4,2] The rats received 0, 5, 50, or 500 mg/kg bw/d of the test material dissolved in water containing 5.3% polyglycol 600 and 4.2% of a 50% aqueous decyl glucoside solution via gavage. Maternal clinical signs of toxicity, body weight, and feed intake were monitored. Dams were killed on gestation day 21. The number of corpora lutea, live and dead fetuses, implantations, and resorptions were recorded. Uterus and fetal weights were also recorded, as were any external abnormalities Half of the fetuses were examined for soft-tissue abnormalities and half for skeletal abnormalities.

No mortality or clinical signs were observed. Blue staining from the test material was observed in body parts and/or feces in 4 of the low-dose group and in all of the animals in the mid- and high-dose groups. Dams of the 500 mg/kg bw/d dose group had decreases in body weights, body weight gain, and corrected body weight gain when compared to controls. These effects were accompanied by reduced feed consumption in some periods. Fetal body weights were decreased at 50 and 500 mg/kg bw/d. Cranial bone ossification was reduced in nearly all high-dose group fetuses and in about one half of the 50 mg/kg bw/d dose group. In the 5 mg/kg bw/d dose group, a generalized reduction in ossification was observed. Incidental cases of malformations were observed in all dose groups including controls (e.g. polydactyly, exencephaly, spina bifida, and abnormal shape of limb bones), but the effects were not dose-related. In the high-dose group, 18 of 166 analyzed fetuses had changes of the major arteries that were attributed to treatment. One fetus in the mid-dose group had persistent truncus arteriosus. The NOAEL for maternal toxicity was 50 mg/kg bw/d, the NOAEL for teratogenicity was 5 mg/kg bw/d, and an NOAEL could not be established for embryotoxicity.[4,5,3,2]

In another developmental study, groups of 22 mated female Wistar rats received Tetrabromophenol Blue (98.8% pure) via gavage on gestation days 6 - 20.[4,5,3] This study was performed in accordance with OECD TG 414. The dams received 0, 3, 30, or 300 mg/kg bw/d of the test material in 5% w/w polyglycol 600, 4% w/w of trade named alkyl polyglucoside containing 50% aqueous decyl glucoside, and 90.5% ultrapure water. The study was carried out in a manner similar to the one described above. No maternal mortalities occurred during the study. No clinical signs or behavioral changes were noted in any dose group. In the mid- and high-dose groups, the feces were bluish in color from gestation day 7 until necropsy. Feed consumption was reduced in the high-dose group throughout the entire treatment period. Consequently, body weight development was reduced in this group from gestation day 8 - 9 onwards, and the mean corrected body weight gain was also reduced. There were no findings in the dams of the low- and mid-dose group that were considered to be treatment-related. The incidence of post-implantation loses and number of fetuses per dam were similar in all groups and not affected by the test material. Mean fetal body weights were reduced in the high-dose group when compared with the control. Compared with the control group, increased incidences of the following findings were observed: cleft palates (1/22 mid-dose and 2/22 high-dose), increased incidences of left-side umbilical arteries and cranially elongated thymuses in the high-dose (number of occurrences not reported), and anophthalmia in the mid-dose group (number of occurrences not reported). There was an increased incidence of fused zygomatic arches in the high-dose group (21 in 12 litters) when compared with the control group (12 in 9 litters). A statistically significant increase in supernumerary rudimentary ribs was observed in the mid- and high-dose groups. No changes were noted in the fetuses of the low-dose group. The maternal NOAEL was determined to be 30 mg/kg bw/d and the embryo-fetal NOAEL was determined to be 3 mg/kg bw/d.

GENOTOXICITY STUDIES

In vitro and in vivo genotoxicity studies on Tetrabromophenol Blue summarized here are detailed in Table 3. In Ames tests at up to 10,000 µg/plate, Tetrabromophenol Blue was not mutagenic, with or without metabolic activation in several strains of *Salmonella typhimurium*.[3,5,4,2,20] Tetrabromophenol Blue was also not genotoxic in *Saccharomyces cerevisiae* strain D_5 in a mitotic recombination assay, with or without metabolic activation, when tested at 10 - 10,000 µg/ml.[20] Genotoxicity was also not observed with Tetrabromophenol Blue in micronucleus assays in human lymphocytes (tested at up to 2250 µg/ml with metabolic activation and up to 1000 µg/ml without metabolic activation),[4,5,3] and in mammalian cell gene mutation tests in L5178Y mouse lymphoma cells (up to 900 µg/ml with metabolic activation and up to 1000 µg/ml without metabolic activation).[20,3,5,4,2] In micronucleus tests in mice injected intraperitoneally with Tetrabromophenol Blue, genotoxicity was not observed at up to 300 mg/kg.[20,3,5,4,2]

CARCINOGENICITY STUDIES

Carcinogenicity studies were not found in the published literature, and unpublished data were not submitted.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

Tetrabromophenol Blue (0.5 g; 96.7 - 98.8% pure) was applied to 3 male New Zealand White rabbits for 4 to 5 h under semi-occlusive dressing.[3,5,4,2] The test material was moistened with 0.25 ml water prior to being applied to clipped skin (150 cm^2). This study was performed in accordance with OECD TG 404. Observations were made 1, 24, 48, and 72 h after application. No skin irritation was observed; however, after 1 h, no scoring of erythema and/or edema was possible in 2 animals due to the staining of the test material on the skin that lasted throughout the observation period. Tetrabromophenol Blue was not considered a skin irritant in this study.

Sensitization

Animal

In a local lymph node assay (LLNA) performed in accordance with OECD TG 429, groups of 5 female CBA/J mice received 0, 0.2, 0.5, 1.5, or 2 % (w/v) Tetrabromophenol Blue (96.7 - 98.8% pure) in dimethyl sulfoxide (DMSO).[3,5,4,2] A negative control group received DMSO alone and a positive control group received *p*-phenylenediamine. A volume of 25 µl of test material was applied to each mouse ear for 3 consecutive days. All animals were sacrificed on day 5. Cell proliferation was assessed by measuring ^{3}H-methyl thymidine incorporation in the cell suspension prepared from the lymph node of each animal. The positive control yielded expected results. The stimulation index (SI) was 0.6, 0.8, 1.0, and 1.1 for the 0.2, 0.5, 1.5, and 2% dose groups, respectively. Tetrabromophenol Blue was not a skin sensitizer in this LLNA.

OCULAR IRRITATION STUDIES

Animal

In an ocular irritation study, 67 mg (~0.1 ml) Tetrabromophenol Blue (96.7 - 98.8% pure) was instilled into 1 eye of each of 3 male New Zealand White rabbits.[3,5,4,2] This study was performed in accordance with OECD TG 405. The eyes were examined at 1, 24, 48, and 72 h after instillation of the test material. The test material caused blue staining of the ocular tissues and of the fur on the head and the paws. The staining prevented scoring of corneal injury, iridial irritation, and conjunctival redness after 1 h, and scoring of the lower eyelid, nictitating membrane, and sclera after 24 h in all animals. Scoring of iridial irritation was hampered by corneal damage (opacity) in 2 animals at 48 and 72 h after instillation. Remnants of the test items were present in the eyes of all animals at 1 and 24 h after instillation. Opacity (maximum grade 4) and epithelial damage (maximum 50% of the corneal area) were observed in addition to iridial irritation (grade 1) in all animals at the 24 or 48 h time period onward. Irritation of the conjunctivae was observed as redness, chemosis, and discharge. Grey/white discoloration of the eyelids (a sign of necrosis) and reduced elasticity of the eyelids were observed in all animals after 48 and 72 h. Based on the severity of the corneal injury, the study was terminated after the 72-h observation period. It was concluded undiluted Tetrabromophenol Blue was corrosive in rabbit eyes.

In another ocular irritation study, 0.1 ml of 2% (w/w) Tetrabromophenol Blue (96.7 - 98.8% pure) solution in phosphate buffer was instilled into one eye each of 3 male New Zealand White rabbits.[3,5,4,2] This study was performed in accordance with OECD TG 405. The eyes were examined at 1, 24, 48, and 72 h. Blue staining by the test material of the fur on the head and paws was noted during the observation period. Instillation of the test material resulted in irritation of the conjunctivae, which was seen as redness and/or discharge. Irritation was completely resolved within 24 h in all animals. No iridial irritation or corneal opacity was observed and treatment of the eyes with 2% fluorescein 24 h after the test material was instilled revealed no corneal epithelial damage in any of the animals. It was concluded the 2% Tetrabromophenol Blue was not an ocular irritant.

MARGIN OF EXPOSURE

MOE is a quantitative factor calculated for cosmetic ingredients by dividing the NOAEL obtained for an ingredient in an animal experiment by the estimated systemic exposure dose (SED) for the ingredient in humans, generally according to US Environmental Protection Agency (EPA) and European Commission SCCS guidelines. The standard MOE value of 100 is derived from multiplying two factors: a 10-fold factor extrapolating data from test animals to human being (interspecies extrapolation) and an additional 10-fold for differences among the human population (intra-species extrapolation). An MOE value greater than 100 has traditionally been considered an indication of safety. The MOE is sometimes referred to as margin of safety (MOS), despite the parameters being definitionally different.

The SCCS calculated an MOE value for 0.2% Tetrabromophenol Blue in a non-oxidative formulation to be 1300.[4,3] Of note, the SCCS also concluded that the approximately 2.5-fold relative increase in the proportion of the two major homologues shown in Figures 1 and 2, in contrast to the homologue composition of study test materials, would not likely influence risk characterization when used as a hair dye in oxidative and non-oxidative products with a final on-head concentration of up to 0.2%. This calculation is based on the adjusted NOAEL of 0.9 mg/kg bw/d (3 mg/kg bw/d from a 90-d oral rat study adjusted with 30% bioavailability) and an SED of 0.00068 mg/kg bw (skin area surface of 580 cm^2 x absorption through skin of 0.07 $\mu g/cm^2$ x 0.001 (unit conversion)/typical human bw of 60 kg).

$$MOE = \frac{NOAEL}{SED} = \frac{0.9 \text{ mg/kg bw/d}}{0.00068 \text{ mg/kg bw/d}} = 1300$$

In an oxidative formulation, the MOE value calculated by the SCCS for 0.2% Tetrabromophenol Blue was 2300.[4,3] The same adjusted NOAEL of 0.9 mg/kg bw/d was utilized in the calculation, but an SED of 0.00039 mg/kg bw was used to reflect the absorption through the skin of an oxidized formulation to be 0.04 $\mu g/cm^2$.

$$MOE = \frac{NOAEL}{SED} = \frac{0.9 \text{ mg/kg bw/d}}{0.00039 \text{ mg/kg bw/d}} = 2300$$

An additional calculation was performed by CIR staff based on the maximum use concentration reported in the Council's updated 2024 survey (0.0025%[9]), the usage of 100 ml of permanent hair dye per application, a retention factor of 0.1, a dermal absorption of 1.2%,[4,5,3] and a NOAEL of 0.9 mg/kg bw/d. This yielded an SED value of 0.00005 mg/kg for a 60 kg adult. The resulting MOE is 18,000.

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. Tetrabromophenol Blue is reported to be used in oxidative and direct hair dye formulations. 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

Tetrabromophenol Blue is reported to function as a hair colorant in cosmetic products. This ingredient is used in oxidative and non-oxidative hair dye formulations. While the ingredient name may give the erroneous impression that there are just 4 bromine substituents, this ingredient most commonly contains 8 bromine substituents (as much as 90% of Tetrabromophenol Blue is octabromo-substituted), with considerably fewer instances with only 7 (and fewer still instances of 6). The full systematic name of this chemical, 4,5,6,7-tetrabromo-3,3-bis(3,5-dibromo-4-hydroxyphenyl)-3H-benzo[c][1,2]oxathiole 1,1-dioxide.

According to RLD that CIR received in 2024, Tetrabromophenol Blue is reported to be used in 40 hair coloring preparations. The 2023 VCRP survey data reported Tetrabromophenol Blue to be used in 2 formulations, 1 hair lightener with color and 1 hair bleach. The results of the concentration of use survey conducted by the Council indicate Tetrabromophenol Blue is used at 0.0025% in hair dyes and colors.

In the European Union, Tetrabromophenol Blue is categorized in Annex III, the list of substances which cosmetic products must not contain except subject to the restrictions laid down. For this ingredient, the regulation states that the maximum concentration applied to the hair must not exceed 0.2% in both oxidative and non-oxidative hair dye products. The SCCS in 2019 concluded that Tetrabromophenol Blue is safe in oxidative and non-oxidative hair coloring products at a final on-head concentration of up to 0.2%.

In a dermal penetration study of oxidative formulation containing 5% Tetrabromophenol Blue in full thickness pig skin, the amount of < 0.339 μg/cm^2 was considered to have passed the skin barrier during a 72-h period (the maximum dermal penetration rate = 0.379 μg/cm^2/72 h). In another study, 2.71 ± 0.89 μg/cm^2 of a formulation containing 0.2% Tetrabromophenol Blue was considered biologically available after 100 mg/cm^2 of the formulation was applied to split thickness pig skin for 60 min. The bioavailability of 0.2% ^{14}C-Tetrabromophenol Blue in non-oxidative and oxidative hair dye formulations was 0.05 ± 0.02 μg/cm^2 and 0.05 ± 0.02 μg/cm^2, respectively.

In toxicokinetics studies, dermal absorption of ^{14}C-Tetrabromophenol Blue in rats was considered poor, with low excretion of the radiolabeled test material via the feces and urine (0.8 and 0.01%, respectively). Oral gavage with the same test material in rats at 10 or 100 mg/kg bw resulted in 107 - 119% of the administered dose recovered in the feces and 0.03-0.1% of the administered dose recovered in the urine. Oral absorption of ^{14}C-Tetrabromophenol Blue was approximately 30% for both doses tested. When this test material was administered intravenously, the mean percent recovery of radioactivity after 96 h was 0.102 ± 0.013% in urine and 112.76 ± 14.30% in feces.

A 28-d oral rat study and a 90-d oral rat study each had an NOAEL for Tetrabromophenol Blue of 3 mg/kg bw/d (for the 90-d study, this value was determined following review of the data by the SCCFNP). For both studies, rats were dosed at up to 100 mg/kg bw/d. In the 28-d study, rats in the high-dose group had an increase in white blood cell counts in the males and increases in cholesterol and glucose in the females. At 10 mg/kg bw/d, only 1 male had a high glucose value. In the 90-d study, chromodacryorrhea was increased in a dose-related manner in females, with behavioral effects being noted in 1 mid- and 2 high-dose animals. Multifocal corneal opacities were observed in 1 male of the 10 mg/kg bw/d group and in 4 males in the 100 mg/kg bw/d group; this effect may have been due to corrosive properties of the test substance and direct eye contact with the fur. In males, changes in platelet values at 100 mg/kg bw/d and in erythrocyte counts, which were statistically significant at 10 and 100 mg/kg bw/d, indicate a hematotoxic potential of Tetrabromophenol Blue. At 100 mg/kg bw/d, changes in urea (males) and cholesterol (female) values were found.

In a rat developmental study of 0, 5, 50, or 500 mg/kg bw/d Tetrabromophenol Blue, the maternal NOAEL was 50 mg/kg bw/d and the NOAEL for teratogenicity was 5 mg/kg bw/d. Dams of the 500 mg/kg bw/d dose group had decreases in body weights, body weight gain, and corrected body weight gain when compared to controls. These effects were accompanied by reduced feed consumption. Fetal body weights were decreased at 50 and 500 mg/kg bw/d. Cranial bone ossification was reduced in nearly all high-dose group fetuses and in about one half of the 50 mg/kg bw/d dose group. In the 5 mg/kg bw/d dose group, a generalized reduction in ossification was observed, thus a NOAEL could not be established for embryotoxicity. In another developmental study in rats, the maternal NOAEL for Tetrabromophenol Blue was 30 mg/kg bw/d and the embryo-fetal NOAEL was determined to be 3 mg/kg bw/d (the maximum dose tested was 300 mg/kg bw/d). Feed consumption was reduced in the high-dose dams throughout the entire treatment period, which resulted in reduced body weight development from gestation day 8 - 9 onwards and reduced mean corrected body weight gain. In fetuses, mean fetal body weights were reduced in the high-dose group, increased incidences of soft-tissue anomalies were observed in mid- and high-dose groups, and increased incidences in skeletal anomalies, including fused zygomatic arches in the high-dose group and an increase in supernumerary rudimentary ribs in the mid- and high-dose groups, were observed.

In Ames tests at up to 10,000 μg/plate, Tetrabromophenol Blue was not mutagenic, with or without metabolic activation in several strains of *S. typhimurium*. Tetrabromophenol Blue was also not genotoxic in *S. cerevisiae* strain D5 in a mitotic recombination assay, with or without metabolic activation, when tested at 10 - 10,000 μg/ml. Genotoxicity was also not observed with Tetrabromophenol Blue in micronucleus assays in human lymphocytes (tested at up to 2250 μg/ml with metabolic activation and up to 1000 μg/ml without metabolic activation), and in mammalian cell gene mutation tests in L5178Y mouse lymphoma cells (up to 900 μg/ml with metabolic activation and up to 1000 μg/ml without metabolic activation). In micronucleus tests in mice injected intraperitoneally with Tetrabromophenol Blue, genotoxicity was not observed at up to 300 mg/kg.

In a dermal irritation study, Tetrabromophenol Blue moistened with water and applied to rabbit skin for 4 to 5 h was not considered a skin irritant. Tetrabromophenol Blue was not a skin sensitizer when tested at 0.2, 0.5, 1.5, or 2% in an LLNA. Tetrabromophenol Blue was corrosive in rabbit eyes when instilled undiluted; however, the test material was not considered an ocular irritant when diluted to 2%.

The SCCS calculated an MOE value for 0.2% Tetrabromophenol Blue as used under non-oxidative conditions to be 1300. This calculation is based on the NOAEL of 0.9 mg/kg bw/d (from a 90-d oral rat study adjusted with 30% bioavailability) and an SED of 0.00068 mg/kg bw (skin area surface of 580 cm^2 x absorption through skin of 0.07 μg/cm^2 x 0.001 (unit conversion)/typical human bw of 60 kg). Under oxidative conditions, the MOE value for 0.2% Tetrabromophenol Blue was calculated by the SCCS to be 2300. The same NOAEL was utilized for the calculation, but the SED of 0.00039 mg/kg bw was used to reflect the absorption through the skin of an oxidized formulation to be 0.04 μg/cm^2. An additional calculation performed by CIR staff using the maximum use concentration of 0.0025% reported in the Council's updated 2024 survey and dermal absorption of 1.2% yielded an MOE of 18,000. The MOE values are greater than 100, a figure generally accepted as the threshold for considering an ingredient safe for use.

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.

Method of manufacturing, acute toxicity, and carcinogenicity studies were not found in the published literature, and unpublished data were not submitted.

DISCUSSION

Tetrabromophenol Blue is reported to function as an oxidative and direct hair dye in hair coloring products at a maximum concentration of 0.0025%. The Panel noted effects were observed in rat developmental and reproductive toxicity studies, but these effects were observed following oral dosing at concentrations much greater than what exposure would be when used in hair dye formulations. The Panel also noted a lack of method of manufacturing, but concerns for this data gap were mitigated by the known purity of this ingredient. The Panel considered these findings, coupled with low dermal absorption, negative genotoxicity, dermal irritation and sensitization, and ocular irritation data, protective MOE values, and the short consumer exposure time to this ingredient in hair dye formulations, and determined that the data are sufficient to conclude that Tetrabromophenol Blue is safe for use as a hair dye ingredient in the present practices of use and concentration.

The Panel recognizes that coal tar hair dye ingredients are exempt from certain provisions of the FD&C 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. However, use of the ingredients in this report as direct hair dyes is not reported.

The Panel's respiratory exposure resource document (available at https://www.cir-safety.org/cir-findings) notes that airbrush technology presents a potential safety concern. Although frequency and/or concentration of use data are now available (and in some cases mandated) for ingredients marketed for use with airbrush delivery systems in certain product categories, no data are available for consumer habits and practices thereof, product particle size, or other relevant particle data (e.g., diameter). As a result of deficiencies in these critical data needs, the data profile is incomplete, and the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Accordingly, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

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

TABLES

Table 1. Chemical properties for the octabromo-homologue of Tetrabromophenol Blue

Property	Value	Reference
Physical Form	Yellowish-grey powder	5,3,4,2
Molecular Weight (g/mol)	985.59	5,3,4,2
Density (g/ml @ 20 ºC)	1.857	4,5,3
Melting Point (ºC)	203-204 (decomposition)	4,5,3,2
Water Solubility (g/l @ 20 ºC & pH 3.5) (% w/v; 98.5% pure; pH 6.2)	0.159 > 25.2	4,5,3 3
Acetone/Water Solubility (weight % @ pH 2.6)	0.9	3,5,4,2
DMSO Solubility (weight %)	> 10	3,5,4,2
log P_{ow} (@ 20 ºC & pH 4.0) (no temperature or pH indicated)	3.71 (estimated by EC Method A.8) 5.98 ± 0.20 (estimated)	4,5,3 4,5,3,2
UV Absorption (λ_{max}) (nm)	224, 299, and 610	5,3,4

Table 2. Frequency (2024/2023) and concentration (2024) of use according to likely duration and exposure and by product category

	# of Uses		Max Conc of Use
	RLD (2024)[7]	VCRP (2023)[8]	% (2024)[9]
Totals*	**40**	**2**	**0.0025**
summarized by likely duration and exposure**			
Duration of Use			
Leave-On	***	*NR*	*NR*
Rinse-Off	***	*2*	*0.0025*
Diluted for (Bath) Use	***	*NR*	*NR*
Exposure Type			
Eye Area	***	NR	NR
Incidental Ingestion	***	NR	NR
Incidental Inhalation-Spray	***	NR	NR
Incidental Inhalation-Powder	***	NR	NR
Dermal Contact	***	NR	NR
Deodorant (underarm)	***	NR	NR
Hair - Non-Coloring	***	NR	NR
Hair-Coloring	***	2	0.0025
Nail	***	NR	NR
Mucous Membrane	***	NR	NR
Baby Products	***	NR	NR
as reported by product category			
Hair Coloring Preparations	***40***		
Hair Dyes/Colors (all types requiring caution statements and patch tests)	20	NR	0.0025
Hair Tints	5	NR	NR
Hair Lighteners with Color	12	1	NR
Hair Bleaches	7	1	NR

NR – not reported

*The total FOU provided for RLD refers to the ingredient count supplied by FDA, and is not a summation of the number of uses per category because each product may be categorized under multiple product categories. For data supplied via the VCRP or by the Council survey, the sum of all exposure types may not equal the sum of total uses because each ingredient may be used in cosmetics with multiple exposure types.

**Likely duration and exposure are derived from VCRP and survey data based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

*** RLD is product-centric and each ingredient may be reported under several product categories, making a summation of RLD misleading in comparison to VCRP data. Accordingly, RLD are presented below by product category (as supplied by FDA), but are not summarized by likely duration and exposure.

Table 3. Genotoxicity studies of Tetrabromophenol Blue

Purity	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
			IN VITRO			
98.6% pure	1 - 5000 µg/plate (1st experiment); 30-3000 µg/plate (2nd experiment)	not reported	*S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	Bacterial reverse mutation test in accordance with OECD TG 471; with and without S9 metabolic activation; positive controls used according to guideline	Not mutagenic; toxicity not stated	4,5,3,2
93.3% pure	up to 5000 µg/plate	DMSO	*S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	Bacterial reverse mutation test in accordance with OECD TG 471; with and without S9 metabolic activation; positive and solvent controls utilized	Not mutagenic; toxicity observed at highest concentrations tested in all strains, with or without metabolic activation; toxicity also observed in TA1535 and TA1537 at lower concentrations	3
95% pure	10-10,000 µg/plate	deionized water	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	Bacterial reverse mutation test; with and without S9 metabolic activation; positive and solvent controls utilized	Not mutagenic, with or without metabolic activation; controls yielded expected results	20
95% pure	10-10,000 µg/ml	deionized water	*S. cerevisiae* strain D_5	Mitotic recombination assay; with and without metabolic activation; positive and solvent controls utilized	Not genotoxic, with or without metabolic activation; little or no toxicity was observed; controls yielded expected results	20
98.8% pure	1000-2250 µg/ml with metabolic activation; 225.3-711.9 µg/ml without metabolic activation	DMSO	human lymphocytes	Micronucleus assay in accordance with OECD TG 487; cultures exposed to mitogen stimulation 24 or 48 h prior to treatment; cultures exposed to test material for 20 h without metabolic activation and for 3 h with metabolic activation; appropriate positive and negative controls used	Not genotoxic. With 24-h mitogen stimulation, no significant increase in frequencies of micronucleated binucleated cells observed at any concentration with or without metabolic activation. With 48-h mitogen stimulation, no induction in micronucleated binucleated cells observed without metabolic activation; however, a slight but statistically significant increase in micronucleated binucleated cells was observed in the intermediate concentration of 1688 µg/ml with metabolic activation. This increase was only observed in one culture and was not concentration-related, therefore was not biologically relevant.	3,5,4
93.3% pure	25-1000 µg/ml with and without metabolic activation for 3 h treatment; 25-400 µg/ml without metabolic activation for 24 h treatment	DMSO	human lymphocytes	Micronucleus assay in accordance with OECD TG 487; cultures exposed to mitogen stimulation 48 h prior to treatment; cultures exposed to test material for 3 h with or without metabolic activation or 24 h without metabolic activation; appropriate positive and negative controls used	Not genotoxic; test material did not induce micronuclei; maximum concentrations analyzed were limited by post-treatment precipitate in the 3 h treatment and/or toxicity in the 24 h treatment (no further details provided); controls yielded expected results	3

Table 3. Genotoxicity studies of Tetrabromophenol Blue

Purity	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
98.6% pure	9-144 µg/ml without metabolic activation for 4 h; 18-288 µg/ml with metabolic activation for 4 h; 18-288 µg/ml without metabolic activation for 24 h	not reported	L5178Y mouse lymphoma cells	Mammalian cell gene mutation test at the thymidine kinase locus in accordance with OECD TG 476; 4-h exposure with and without metabolic activation and 24-h exposure without metabolic activation; appropriate negative and positive controls used	Not genotoxic. Toxicity observed from 144 µg/ml without metabolic activation and from 288 µg/ml with metabolic activation. After 4 h, the test material induced a dose-related significant increase of small colony mutants without metabolic activation; however, this effect was not repeated in the 24-h treatment, and re-evaluation by the SCCS in 2012 determined the increases were minor and of no biological relevance. With metabolic activation, an increase of the induction of small colony mutants was observed at the highest dose, which was also determined to not be biologically relevant by the SCCS in 2012. Controls yielded expected results.	4,5,3,2
95% pure	100-900 µg/ml with metabolic activation; 500-1000 µg/ml without metabolic activation	deionized water	L5178Y mouse lymphoma cells	Mammalian cell gene mutation test at the thymidine kinase locus; 4-h exposure with and without metabolic activation; positive and solvent controls utilized	Not mutagenic, with or without metabolic activation; moderate toxicities induced (77.5 - 24.6% relative growths, no further details provided) without metabolic activation, but the test material was insoluble at higher concentrations; with metabolic activation, a wide range of toxicities were induced 50.2 - 11.1% relative growths, no further details provided); controls yielded expected results	20
			IN VIVO			
98.6% pure	75, 150, or 300 mg/kg	water	groups of 5 male and 5 female NMRI mice	Mammalian bone marrow micronucleus test in accordance with OECD TG 474; animals received test material intraperitoneally; mice were killed 24 h after treatment, an additional high-dose group of mice were killed 48 h after treatment; appropriate positive and negative controls used	Not clastogenic/aneugenic; test material did not induce micronuclei but some reduction of the polychromatic erythrocyte/normal erythrocyte ratio was observed in the treated animals; controls yielded expected results; in preliminary tests up to 400 mg/kg, toxic effects were observed	2,5,3,4
95% pure	24, 80, or 240 mg/kg	0.5% carboxymethylcellulose	groups of 5 male and 5 female CD-1 mice	Mammalian bone marrow micronucleus assay; mice received test material via intraperitoneal injection; animals were killed 24 and 48 h after administration of the test article and 24 h after administration of the positive and negative controls	Not genotoxic; test material did not induce significant increases in micronucleus frequencies in bone marrow polychromatic erythrocytes; controls yielded expected results	20

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