# Final Report on the Safety Assessment of Basic Violet 1, Basic Violet 3, and Basic Violet 4

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**Abstract**

Basic Violet 3, Basic Violet 1, and Basic Violet 4 are triphenylmethane dyes that function as direct (nonoxidative) hair colorants. No current uses or use concentrations in cosmetics are reported. The term *Gentian Violet* is used synonymously with *Basic Violet 1* and *Basic Violet 3*, although the chemical structures of these 2 dyes are not the same. The Cosmetic Ingredient Review Expert Panel noted that Basic Violet 1, 3, and 4 contain quaternary ammonium ions, and therefore the rate of penetration across the epidermis is expected to be slow. The panel concluded that because of the carcinogenic potential of these dyes, insufficient data exist to support the safety of Basic Violet 1, 3, and 4 in cosmetic formulation. Dermal absorption data and a risk assessment are needed to complete this safety assessment.

**Keywords**

cosmetics, safety, basic violet 1, basic violet 3, basic violet 4

Basic Violet 3 is a hair colorant with only limited use in cosmetics. It was selected for review based on information in the scientific literature that raised concerns over possible developmental toxicity, mutagenesis/carcinogenesis, and other toxicity. Basic Violet 1 and Basic Violet 4 are included in this scientific literature review because of their structural similarities and their common use as hair colorants. Although there were almost no data on Basic Violet 4, it may be that the similarity in structure and use will allow extrapolation of data on Basic Violet 3 and Basic Violet 1. All are direct (nonoxidative) hair dyes.

Gentian Violet is a synonym of Basic Violet 1, and Quinby[1] defines Gentian Violet as "a partially purified mixture of at least three rosaline dyes, mainly crystal violet." In the literature, Gentian Violet is often used synonymously with both Methyl Violet (Basic Violet 1) and Crystal Violet (Basic Violet 3). Because it is frequently the studied chemical, subheadings will be included for Gentian Violet, with the understanding that it may be either Basic Violet 1 or Basic Violet 3. If a chemical structure given in a study made it clear that the chemical studied was Basic Violet 1 or Basic Violet 3, the compound was categorized as such.

## Chemistry

### Definition and Structure

*Basic Violet 1.* As given in the *International Cosmetic Ingredient Dictionary and Handbook*,[2] Basic Violet 1 (CAS No. 8004-87-3) is a triphenylmethane color that is a mixture of methylated pararosanilines consisting principally of the tetramethyl, pentamethyl, and hexamethyl derivatives. The pentamethyl derivative of Basic Violet 1 conforms to the structure in Figure 1.[3]

Basic Violet 1 has the following synonyms[4]:

- Gentian Violet
- Hexamethyl-p-rosaniline chloride
- Methyl Violet

The *International Cosmetic Ingredient Dictionary and Handbook*[2] also lists a color index number, CI 42535, as a synonym.

*Basic Violet 3.* As given in the *International Cosmetic Ingredient Dictionary and Handbook*,[2] Basic Violet 3 (CAS No. 548-62-9) is a triphenylmethane color that conforms to the structure in Figure 2.

As given by the Registry of Toxic Effects of Chemical Substance (RTECS),[5] Basic Violet 3 has the following synonyms:

- Ammonium, (4-(bis(p-(dimethylamino)phenyl)-methylene)-2,5-cyclohexadien-1-ylidene)dimethyl-,chloride
- Aniline Violet
- Bismuth Violet

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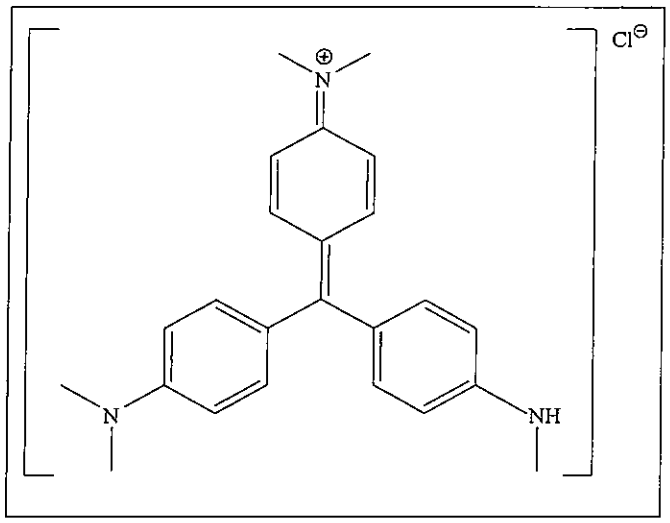

**Figure 1.** Basic Violet 1.

- Crystal Violet
- Gentian Violet
- Hexamethyl p-rosaniline hydrochloride
- Hexamethyl Violet
- Hexamethyl-p-rosaniline chloride
- Methanaminium, -(4-(bis(4-(dimethylamino)phenyl)-methylene)-2,5-cyclohexadien-1-ylidene)-methyl, chloride

Gottschalck and Bailey also list the color index, CI 42555[2]:

- N-[4-[bis[4-(dimethylamino)phenyl]-methylene]-2, 5-cyclohexadien-1-ylidene]-N
- Methylmethanaminium chloride
- Methylrosanilinium chloride

*Basic Violet 4.* Basic Violet 4 (CAS No. 2390-59-2) is a triphenylmethane color that conforms to the structure depicted in Figure 3.[2]

According to RTECS, Basic Violet 4 has the following synonyms[6]:

- [4-bis[4-(diethylamino)phenyl]-methylene]2,5-cyclohexadien-1-ylidene]diethylammonium chloride
- Ethanaminium, N-(4-(bis(4-(diethylamino) phenyl)methylene)-2,5-cyclohexadien-1-ylidene)-N-ethyl-,chloride
- Ethyl Violet

Gentian Violet is defined by Quinby[1] as "a partially purified mixture of at least three rosaline dyes, mainly crystal violet."

## Chemical and Physical Properties

Basic Violet 1 has a molecular weight of 392.95.[3] Basic Violet 3 has a molecular weight of 408.00.[7] Basic Violet 4 has a molecular weight of 492.15.[8] No other chemical or physical properties were available.

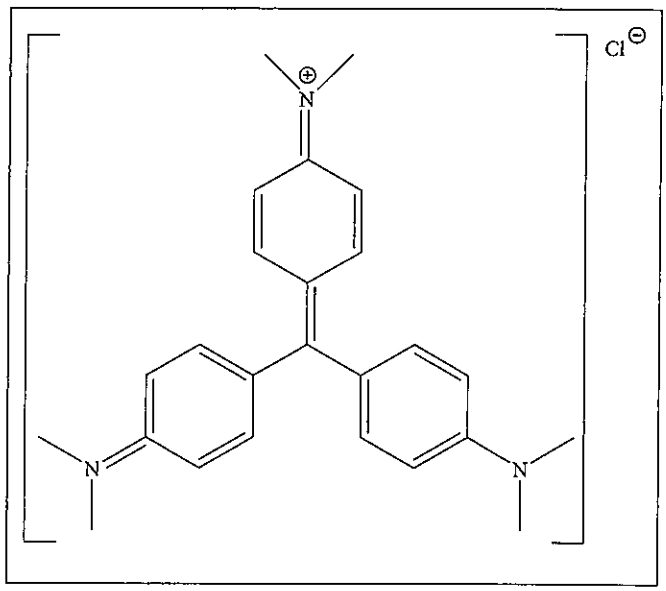

**Figure 2.** Basic Violet 2.

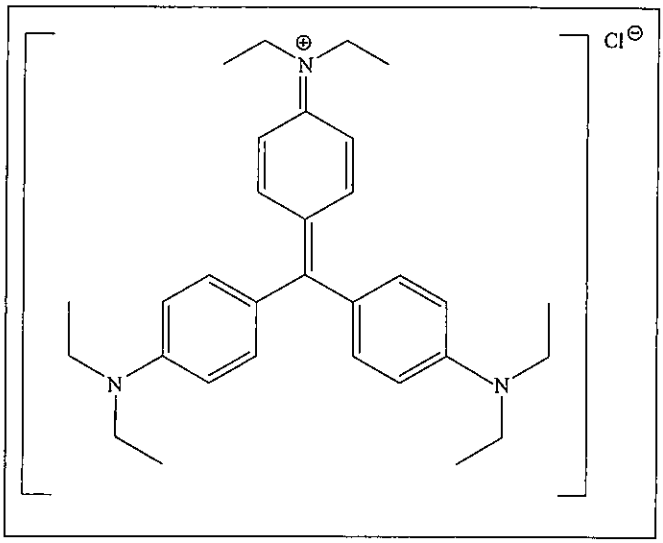

**Figure 3.** Basic Violet 4.

## Methods of Manufacture

Commercial preparations of Gentian Violet are described by Docampo and Moreno.[9] The dyes are manufactured by oxidizing a mixture of aniline and p-toluidine, the oxidizing agent most commonly used being arsenic acid. Nitrobenzene has also been used. Pure Basic Violet 3 has been made by the action of carbonyl chloride (phosgene) on N,N-dimethylaniline.

## Analytical Methods

Thin-layer chromatography,[10] reverse-phase high-pressure liquid chromatography,[11] a modified liquid chromatography,[12] and a spectrophotometric procedure[13] have been used to detect Basic Violet 1 and Basic Violet 3.

### Impurities

Pharmaceutical grades of Basic Violet 3 have been purified to reduce the heavy metal salt content below the limits of 10 ppm for arsenic and 30 ppm for lead.[9] Although the term *Gentian Violet* is used, the structure given is for Basic Violet 3.

## Use

### Cosmetic

Basic Violet 1, Basic Violet 3, and Basic Violet 4 are color additives that function as nonoxidative hair colorants.[2] Under the US Food and Drug Administration (FDA) voluntary cosmetic registration program (VCRP), manufacturers provide information on their use of individual cosmetic ingredients as a function of product type. No current uses of any of these ingredients are reported in the VCRP.[14]

Although an industry survey of current use concentrations reported use of Basic Violet 4, the Cosmetic, Toiletry, and Fragrance Association (CTFA) reports that the product is no longer being produced.[15]

Basic Violet 1, Basic Violet 3, and Basic Violet 4 are considered coal tar hair dyes 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 Federal Food, Drug, and Cosmetic Act. To be exempt, the following caution statement must be displayed on all coal tar hair dye products:

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

Product labels shall also bear a caution statement and patch test instructions for determining whether the product causes skin irritation. The Cosmetic Ingredient Review (CIR) Expert Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 hours after application of the test material and prior to the use of a hair dye formulation.

In regulations regarding cosmetics in the European Union, Basic Violet 1, Basic Violet 3, and Basic Violet 4 have been banned for use in hair dyes.[16]

### Noncosmetic

Basic Violet 1 is used as an acid-base indicator (acids, yellow; bases, violet), a textile dye, a human medicine (topical antibacterial and anti-allergen), an alcohol denaturant, and a biological stain.[17,18] It is classified as a class I medical device for its use as a dye and stain.[19]

Basic Violet 3 also is used as an acid-base indicator (pH 0, green; to pH 2.0, blue), a bacteria stain, and an antiseptic.[17] It is classified as a class I medical device for its use as a dye and stain.[20]

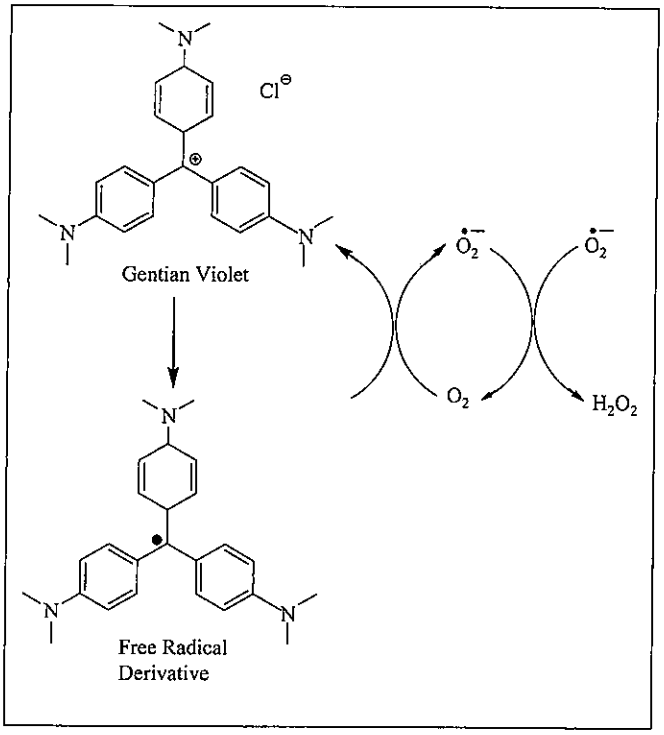


**Figure 4.** Reductive metabolism of Gentian Violet.[22]

Basic Violet 4 also is used as a dye for textile, leather, paper, and printing inks.[21]

FDA has determined that neither Basic Violet 1 nor Basic Violet 3 is generally recognized as safe for use in animal feed or safe and effective for any veterinary drug use (21CFR500.29 and 21CFR500.30).

## General Biology

### Photoreduction Reactions

Docampo et al[22] studied the photodynamic action of Gentian Violet in *Trypanosoma cruzi.*

Visible light causes photoreduction of Gentian Violet to a carbon-centered radical. Under aerobic conditions, this free radical autooxidizes, generating a superoxide anion whose dismutation yields hydrogen peroxide. This photodynamic action of Gentian Violet is thus probably mediated by the oxygen reduction products. The reductive metabolism of Gentian Violet is shown in Figure 4.[22]

### Cytotoxicity

*Basic Violet 1.* Norrby and Mobacken[23] studied the effects of Basic Violet 1 on the proliferation of a human fibroblast-like cell line (GP 119-2). Basic Violet 1 (tested at a concentration of 10 μg/mL) was strongly cytotoxic.

*Basic Violet 3.* Norrby and Mobacken[23] studied the effects of Basic Violet 3 (tested at a concentration of 10 μg/mL) on the proliferation of human fibroblast-like cell line (GP 119-2). Basic Violet 3 was strongly cytotoxic. In an epithelial-like cell line, HeLa cells, Basic Violet 3 (tested at a concentration of 1- 100 μg/mL) was cytotoxic, but less so than when using fibroblast-like cells.

*Gentian Violet.* Rosenkranz and Carr[24] exposed normal and mutant *Escherichia coli* (lacking DNA polymerase Pol $A^-$) to Gentian Violet. The mutant *E coli* was more sensitive to Gentian Violet, Crystal Violet, and Methyl Violet. Wolfe[25] demonstrated that Crystal Violet inhibits DNA synthesis catalyzed by *E coli* B polymerase I.

## Animal Toxicology

### Acute

*Basic Violet 1.* Oelsner[26] performed acute oral and intraperitoneal toxicity studies of Basic Violet 1, which made up 28% of an indelible pencil. Pencil leads were ground into a fine powder, and 3% to 5% lead suspensions in water were used without having been filtered from the undissolved matter. For the oral study, 17 young and 12 adult rats (strain and sex not given) were used. Although the experimental doses for Basic Violet 1 are not given, the author states that the oral median lethal dose ($LD_{50}$) was greater than 2160 mg/kg for young rats and greater than 2600 mg/kg for adult rats. For the intraperitoneal study, 21 young rats and 12 adult rats were administered the suspension (volume and effective dose not given). The intraperitoneal $LD_{50}$ was 64 mg/kg for young rats and 57 mg/kg for adult rats. The author noted that the difference was slight and therefore did not justify inference of lower sensitivity of the young rats. The signs of toxicity in the rats were not specified, although the cause of death was cited as exudative peritonitis due to the highly local irritative action of "crystal violet."

*Basic Violet 3.* Oelsner[26] performed acute oral and intraperitoneal toxicity studies of Basic Violet 3. For the oral study, 44 young and 24 adult rats (strain and sex not given) were used. Animals received a 1% or 2% solution of Basic Violet 3 in water by gavage. The author states that the oral $LD_{50}$ was approximately 90 mg/kg for young rats and approximately 650 mg/kg for adult rats. For the intraperitoneal study, 36 young and 16 adult rats were given 0.2% Basic Violet 3 (volume not given). The intraperitoneal $LD_{50}$ was 17 mg/kg for both young and adult rats.

*Basic Violet 4.* Bushy Run Research Center[27] conducted an acute oral toxicity study of Basic Violet 4 mixed with acetic acid (proportions not given) in male and female Sprague-Dawley rats. There were 5 rats of each sex for each dose group. Male rats received a 250-, 500-, 595-, 707-, or 1000-mg/kg dose, whereas female rats received a 62.5-, 125-, 250-, 500-, or 1000-mg/kg dose by gavage. The $LD_{50}$ was 549 mg/kg in male rats and 308 mg/kg in females. Signs of toxicity included sluggishness, unsteady gait, red discharge around the nose, tremors, diarrhea, emaciation, and a moribund appearance.

### Short-Term Oral Toxicity

*Basic Violet 3.* The National Center for Toxicological Research (NCTR)[28] studied the teratogenicity and toxicity of Basic Violet 3 in CD rats. (The teratogenicity data are discussed in the Reproductive and Developmental Toxicity section.) Female rats (n = 153) were mated with 127 breeder males and dosed daily via gavage with Basic Violet 3 at doses of 0.0, 2.5, 5.0, and 10.0 mg/kg/d on gestational days 6 through 15. Dams were observed for clinical signs of toxicity. On gestational day 20, dams were killed and body and liver weights were recorded.

The maternal mortality rate was 9.4% in the 10.0-mg/kg group. All other dams from all other dose groups survived until termination of the experiment. A significant trend for reduced maternal body weight was found on gestational days 11 and 15, with the value for the 10-mg/kg group significantly different from controls on gestational day 11. A significant trend toward reductions in maternal weight gain for the gestation period and treatment period and absolute weight gain were found with the values for these 3 parameters in the 10.0-mg/kg group significantly below those of controls. Clinical signs of toxicity were dose dependent and included weight loss of more than 5 grams in 24 hours, wheezing, lethargy, weakness, diarrhea, lacrimation, and rough coat.[28]

The NCTR[29] evaluated the teratogenicity and toxicity of Basic Violet 3 in New Zealand white rabbits. (The teratogenicity data are discussed in the Reproductive and Developmental Toxicity section.) Females were artificially inseminated and dosed by gavage on gestational days 6 through 19 with Basic Violet 3 (0, 0.5, 1.0, and 2.0 mg/mL) with distilled water as the vehicle. A dose volume of 1 mL per kilogram of body weight was used. Dams were weighed on gestational days 0, 6 to 19 (prior to daily dosing), and 30 (prior to being killed) and were also observed for clinical signs of toxicity. Rabbits that died between gestational day 0 and study termination were necropsied. On gestational day 30, approximately 1.5 days before expected parturition, pregnant does were killed. Clinical signs of toxicity and body and liver weights were recorded.

The maternal mortality was 0.0% in the control group, 7.4% in the 0.5-mg/mL group, 15.4% in the 1.0-mg/mL group, and 22.6% in the 2.0-mg/mL group. A significant trend was seen toward a reduction in maternal body weight on gestational day 19 (end of dosing) and in maternal weight gain (gestational period and treatment period). For maternal weight gain, all Basic Violet 3–exposed groups were significantly lower than for controls for both treatment and gestation period. Dose-related clinical signs included wheezing, diarrhea, congestion, wet nose, dyspnea, lacrimation, anorexia, and cyanosis.[29]

### Ocular Irritation

*Basic Violet 3.* Ballantyne et al[30] performed what was described as a standard rabbit eye irritation test using 20 mg/

mL of Basic Violet 3 in water. Basic Violet 3 rapidly produced severe and persistent blepharitis with hyperemia, edema, and necrosis of the conjunctivae and nictitating membrane. Mild keratitis was apparent within 24 hours and over the following 21 days became progressively more severe. At 3 weeks, there was gross opacification, deformity, and vascularization of the cornea. Keratitis often obscured a severe iritis. Studies with more dilute solutions demonstrated the damaging effects to be concentration dependent. The no-effects concentration was approximately 0.25 mg/mL.

*Gentian Violet.* Dhir et al[31] instilled 1% Gentian Violet in the conjunctival sac of 2 rabbits 3 times a day. The total number of days was not reported. Both rabbits developed conjunctival congestion and discharge on the following day. After 3 days of instillation, there was some necrosis of the conjunctiva. Conjunctival biopsies had variable thinning of epithelial lining with total loss of goblet cells and subepithelial capillary congestion with neutrophillic infiltration.

### Other Animal Toxicity

No animal dermal irritation, sensitization, or phototoxicity studies were found for any of the ingredients.

### Reproductive and Developmental Toxicity

*Basic Violet 3.* The NCTR[28] studied the teratogenicity of 0 to 10 mg/kg Basic Violet 3 in CD rats. (For a description of the methods of this study, see the Short-Term Toxicity section.) Uterine implantation sites, resorptions, dead fetuses, and live fetuses were evaluated. Fetuses were examined for visceral and skeletal malformations. No dose-related differences were observed in the following reproductive measures: number of implantation sites per litter; number or percentage of resorptions, fetal deaths or nonlive per litter. Among live litters, the number of live fetuses per litter, sex of fetuses, average fetal body weight, and average male or female fetal body weight per litter also were unaffected. There was a significant trend toward an increased number and percent of pups affected (nonlive plus malformed) per litter with dose. The number of litters with affected fetuses was significantly increased in the 10.0-mg/kg group versus controls. No gross malformations were observed in any dose group. Major visceral malformations included hydronephrosis (left or right) and/or hydroureter (unilaterally or bilaterally). Skeletal defects consisted mainly of short rib involving the 13th rib unilaterally or bilaterally. When the incidence of all malformations was analyzed, there was a significant trend across dose groups toward increased number and percentage of fetuses, both males and females, per litter. The number and percentage of malformed fetuses, both males and females, per litter was significantly increased in the 10.0-mg/kg group versus controls as was the number of litters with malformed fetuses. Because there was no significant incidence of malformations in the lower dose groups, the authors concluded that fetal response to Basic Violet 3 may be due to the compromised status of the dam.[28]

The NCTR[29] also evaluated the teratogenicity of 0 to 2 mg/kg Basic Violet 3 in New Zealand white rabbits. (For a description of the methods of this study, see the Short-Term Toxicity section.) Uterine implantation sites, resorptions, dead fetuses, and live fetuses were recorded. Fetuses were also examined for visceral and skeletal malformations.

All Basic Violet 3–exposed groups had a significant increase in the number of implantation sites per litter versus controls. Percentage of resorptions per litter and number of litters with resorptions, as well as the percentage of nonlive (dead plus resorbed) and affected (nonlive plus malformed) pups per litter, exhibited a dose-related upward trend. For live litters, the number of fetuses (male and/or female) per litter did not differ among dose groups. Average fetal body weight per litter had a significant downward trend with all dose group values significantly lower than controls. When separated by sex, only female fetal body weight per litter exhibited a significant downward trend. There were no significant dose-related effects on the incidence of gross, visceral, or skeletal malformations per litter or in the number (or percentage) of fetuses, sex of the fetuses, number malformed per litter, or percentage of litters with malformed fetuses.[29]

## Genotoxicity

### In Vitro

*Basic Violet 1.* Bonin et al[32] tested the mutagenicity of 22 dyes, including Basic Violet 1 (0, 0.32, 1.0, 3.2, and 10 μg per plate) using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 in the Ames assay with and without S9. Positive controls were 2-acetylaminofluorene and β-propiolactone. Basic Violet 1 was mutagenic only in TA1535 at low chemical concentrations in the absence of S9.

Chung et al[33] tested the mutagenicity of 17 dyes, including Basic Violet 1 using *S typhimurium* strains TA98, TA1535, TA1537, and TA1538 in the Ames assay (concentrations of Basic Violet 1 not given) with and without S9. Controls were dimethyl sulfoxide (DMSO), sodium azide, 2-nitrofluorene, and 2-aminoanthracene. Basic Violet 1 was not mutagenic (no data given).

Kvelland[34] studied the mutagenic effect of Basic Violet 1 in phage T4D. Revertants of $r^+$ particles in phage stocks and in phage lysates were scored by plating on K12, and total phage particles, on *E coli* B cells. Five minutes after infection, the phage-infected bacterial culture was superinfected by the same phage (multiplicity of 5) to produce lysis inhibition. After 10 minutes, Basic Violet 1 was added at concentrations of 1.8 to 400 μg/mL. Incubation continued for 30 minutes, and then the suspension was diluted and incubated for another 60 minutes. Control phages were treated in the same way, except that Basic Violet 1 was omitted. The concentrations 3.3, 9.5, 18.1, 91, 130, and 200 μg/mL Basic Violet 1 gave mutation frequencies significantly higher than the control. Concentrations

of Basic Violet 1 higher than 200 μg/mL did not significantly increase the mutation frequency, and doses higher than 91 μg/mL drastically reduced the yield of phages.

*Basic Violet 3.* Au et al[35] studied the chromosome damage produced by Basic Violet 3 using Chinese hamster ovary (CHO) cells. When a comparison was made between 6 Basic Violet 3 samples (concentration not given) by exposing the cells for 5 hours, conflicting results were found. For Basic Violet 3, damage ranged from 2.60 to 5.84 chromosome breaks per cell. The controls had 0.15 breaks per cell.

Wakelin et al[36] reported that when Basic Violet 3 binds to closed circular DNA, the helix becomes unwound.

Bonin et al[32] tested the mutagenicity of 22 dyes, including Basic Violet 3 (0, 0.1, 0.32, 1.0, and 3.2 μg per plate) using *S typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 in the Ames assay with and without S9. Positive controls were 2-acetylaminofluorene and β-propiolactone. Basic Violet 3 was mutagenic only in TA1535 in the absence of S9.

Levin et al[37] studied the effect of light on genetic toxicity of Basic Violet 3 and the effect of rat liver S9 fraction on its genetic toxicity in the Rosenkranz assay and in 3 strains of *Salmonella.* The wild-type strain (W3110) and its polymerase A–deficient mutant (p3478) were used. Cultures with benzo[a]-pyrene, methyl methanesulphonate, and chloramphenicol served as positive controls. Plates were then incubated in the dark, or under a combination of fluorescent (15 W) and incandescent lights (40 W), at 37°C for 24 hours. The diameters of the zones of inhibition around the paper discs were measured. Log-phase culture (2.0 mL) was incubated at 37°C for 30 minutes with 10 μL of Basic Violet 3 (0.1, 0.5, 1.0, 5.0, and 10.0 mg/mL in water) and then dispensed into Petri plates that were maintained in the dark or irradiated (500 W) for 3 minutes. A $10^4$ dilution of the cultures was then spread onto HA (undefined) medium and incubated at 37°C for 24 hours.

The inhibition of growth of the wild-type and mutant *E coli* showed a dose-response relationship to Basic Violet 3 concentration. A genotoxic effect was also observed under conditions of dark incubation for all concentrations of Basic Violet 3 tested. This effect was enhanced under conditions of light incubation. The presence or absence of S9 had no effect. The authors acknowledged the possibility that a photoproduct of Basic Violet 3 might diffuse more rapidly than the parent compound, thus giving an increased zone of inhibition without necessarily having an increased genetic toxicity. Therefore, a parallel study was conducted in which the number of viable cells surviving treatment in a liquid medium was determined. From this, it was clear that the genetic toxicity of Basic Violet 3 was enhanced by irradiation with visible light.

*S typhimurium* strains TA98, TA100, and TA1537 were used in the Ames test. Basic Violet 3 (in water) was added in concentrations of 1.0, 5.0, 10.0, and 50.0 μg per plate with and without S9. Benzo[a]pyrene was the positive control. After incubation at 37°C for 48 hours either under conditions of illumination or in the dark (same as above), plates were then scored for $His^+$ revertants. Basic Violet 3 was nonmutagenic for any of the 3 strains tested. At concentrations above 5.0 μg per plate, it was sufficiently toxic to sterilize the plate under conditions of dark incubation.[37]

Thomas and MacPhee[38] studied the mutagenicity of Basic Violet 3 with the Ames assay using *S typhimurium* strain TA1535 and, with strain DG1669, an *E coli* K12 derivative carrying the *lacZ*(ICR24) frameshift marker that is DNA repair proficient. Mutagenicity tests were carried out using Basic Violet 3 (dissolved in water) at concentrations of 0.25, 0.05, 0.1, and 0.5 μg per plate for TA1535 and 25, 50, 75, and 100 μg per plate for DG1669. No positive controls were reported. In strain TA1535, no significant mutagenic effects of Basic Violet 3 were observed at 0.025 to 0.5 μg per plate, although the compound was detoxified significantly when S9 mix was present. By contrast, the experiment with DG1669 showed that Basic Violet 3 was a mutagen, causing frameshift mutations in repair-proficient bacteria. Mutagenicity was apparent with and without metabolic activation, although the response was much more obvious on the plates containing S9 mix, probably because of detoxification. At doses of 75 and 100 μg per plate, without S9, the Basic Violet 3 concentration was sufficient to kill a high proportion of the cells; thus, significantly lower mutant numbers were observed.

Tacal and Özer[39] studied the formation of colorless adducts with human serum proteins by cationic triarylmethane dyes including Basic Violet 3 (concentration not given) spectrophotometrically at 25°C, in 50 mM 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (pH 8), by monitoring the loss in color in the absence and presence of human serum proteins as potential addends. Unfractionated serum caused a rapid bleaching of methyl green and malachite green, whereas pararosaniline and Basic Violet 3 were unaffected. Basic Violet 3 thus reportedly had a lower tendency to form adducts.

*Gentian Violet.* Au et al[40] studied the genetic toxicity of Gentian Violet with the Ames and the Rosenkranz bacterial assays as well as the CHO, chicken embryo, and mouse bone marrow cells assays. The *S typhimurium* strains TA1535, TA1537, TA98, and TA100 were treated with 0.1 to 50 μg of Gentian Violet. Cultures with 2-aminoanthracene were used as positive controls. Without the S9 mixture, Gentian Violet was bactericidal at doses of 10 μg and higher. When S-9 mixture was present, the purple color of the dye was converted to pink and it lost its toxicity. Only slight toxicity was detected at 50 μg per plate. Gentian Violet was not found to be toxic in an Ames assay but did cause reparable DNA damage in a Rosenkranz assay.

Cultures of wild-type *E coli* (*E coli* W3110 *pol* $A^+$) and the mutant (*E coli* p3478 $A^-$) were added to 3 mL of soft agar (0.75%) and the mixtures poured onto the surface of a hard (1.5%) agar plate (25 mL). Gentian Violet was added at concentrations of 1, 10, 25, and 1000 μg per plate with or without the simultaneous addition of S9 mixture to the overlay agar. Cultures treated with ethyl methanesulfonate, N-methyl-N′-nitro-N-nitrosoguanidine, and streptomycin were used as controls. The inhibition of growth of the wild-type and mutant

*E coli* showed a dose-response relationship to Gentian Violet treatment. The inhibition of the mutant cells was consistently higher than that of the wild-type. Although the inhibitory activity of the Gentian Violet was reduced by S-9 in both the wild-type and the mutant cultures, it was not eliminated.

CHO cell cultures were treated with 0.5 mL of 5, 10, and 20 μg/mL of Gentian Violet with or without the presence of S-9 mixture. The experiment was done twice. The presence of S-9 mixture alone did not induce any increase in chromosome damage compared with the untreated control. The damage induced by 5 μg/mL of Gentian Violet (3.71 breaks per cell) was reduced to the control level (0.23 breaks per cell) in the presence of S-9 mixture (0.56 breaks per cell). When the concentration of Gentian Violet was increased to 10 μg/mL without the proportional increase in S-9 mixture, induction of chromosome damage was observed again (0.75 breaks per cell). When the concentration was increased to 20 μg/mL, chromosomes were too damaged to analyze.

The mutagenicity of Gentian Violet in the *S typhimurium* assay was reevaluated using TA98, TA100, and 2 tester strains, TA97 and TA104, using100 μL of the test chemical (0, 0.1, 0.25, 0.5, 1.0, 25, 5.0, 10.0 μg per plate) or solvent (dimethyl sulfoxide). Methylmethane sulfonate (MMS) and benzo(a)pyrene (BP) were the positive controls.[41] Gentian Violet appeared to be mutagenic in strain TA97 with and without S9 activation and in strain TA104 with S9. The strongest mutagenic responses were found in strain TA97 in the presence of S9. Gentian Violet produced dose-responsive increases in mutagenicity up to a concentration of approximately 0.5 μg per plate where in excess of 200 revertants per plate over the solvent controls were induced. Without S9, 0.1 to 0.5 μg per plate of Gentian Violet produced increased numbers of revertants per plate in TA97, but no dose response was seen. Gentian Violet also produced a positive, dose-responsive increase in reversions in strain TA104 in the presence of S9, reaching a maximum of more than 300 revertants per plate over the solvent controls at a concentration of 5 μg per plate. Weak mutagenic responses were found in TA100, and Gentian Violet was nonmutagenic in TA98 and TA104 without S9. At higher doses without S9, Gentian Violet was toxic and killed a large portion of the TA97 cells, and fewer mutant colonies were observed. However, at the same doses with S9, Gentian Violet was detoxified and mutant yield was increased.

Gentian Violet was assayed for mutagenicity in 2 CHO cell strains, CHO-K1-$BH_4$ and CHO-AS52. Whereas CHO-K1-$BH_4$ cells measure gene mutations at the *hprt* locus, CHO-AS52 cells measure mutations using the *xprt* or *gpt* locus. Cultures were exposed to 0 to 1.5 μg/mL Gentian Violet for 5 hours in a nutrient mixture. Assays were conducted in the presence or absence of an S9 activation system. Cells were then incubated for 7 days and plated. All cultures were incubated for 7 days before being fixed, stained, and counted. Results were expressed as 6-thioguanine–resistant mutants/$10^6$ clonable cells. At the concentrations tested, no mutagenicity was seen in CHO-K1-$BH_4$ cells, whereas 66 mutants per $10^6$ clonable cells were obtained in CHO-AS52 cells at a Gentian Violet concentration producing a high level of cytotoxicity. This positive response, however, was not consistently found in subsequent experiments. Toxicity was apparent in CHO cells, both with and without S9, although it was more evident in assays conducted in the absence of metabolic activation.

### *In Vivo*

*Gentian Violet.* Gentian Violet was dissolved in water and applied to the inner shell membrane of 74-hour Cornell K-strain chicken embryos at dosages of 0.5, 2.0, 5.0, 10.0, 20.0, 100, 1000, and 2000 μg per embryo. Embryos grew in the presence of 0.5 to 10 μg of Gentian Violet over the experimental period of 22 hours but were colored purple by this dye. No increase in the rate of sister chromatid exchange (SCE) above control level was observed in these embryos. At dosages of 20, 100, 1000, and 2000 μg, Gentian Violet was very toxic by completely inhibiting growth. Only one embryo survived at 100 μg, and it did not show a significant increase in SCE. No signs of chromosome breakage were seen in embryos exposed to 0.5 to 10 μg of Gentian Violet.

Swiss albino mice (4-6 per cage, total number not given, sex not given) were given water containing concentrations of Gentian Violet at 20 or 40 μg/mL. Animals were chosen weekly at random for 4 weeks and killed for chromosome analysis of the bone marrow. Bone marrow from both femurs was isolated, and mitotic index was scored as the percentage of mitotic cells observed in 1000 cells. The final dosage was calculated to be approximately 4 and 8 mg/kg/d Gentian Violet for each mouse. It was observed that the amount of chromosome damage was not significantly different for the 2 doses for the initial 3 weeks (2.93% and 2.13% for 20 μg/mL and 40 μg/mL, respectively). The mitotic index was decreased by the fourth week (0.2% and 0.2%), but there was no significant increase in chromosome damage other than a general toxicity.[40]

To compare the in vivo and in vitro DNA-damaging potential of Gentian Violet, spleen lymphocytes from $B6C3F_1$ mice were exposed both in vivo and in vitro to Gentian Violet. For in vivo experiments, lymphocytes were isolated from animals pretreated for 1 hour with 0 to 10 μg/mL Gentian Violet injected into the tail vein. Animals treated with MMS were used as positive controls. For in vitro studies, lymphocytes isolated from untreated animals were exposed to 0 to 1.0 μg/mL Gentian Violet. No DNA damage was detected by nucleoid sedimentation analysis of lymphocytes.

SV40-transformed Chinese hamster embryo C060 cells were exposed in vitro to Gentian Violet. Cells were seeded into culture flasks. After 24 hours, cells were treated with 0.02, 0.05, or 0.125 μg/mL Gentian Violet, DMSO (vehicle control), or 0.1 μg/mL DMBA (DMBA is dimethylbenzanthracene undefined; positive control). In C060 cells, Gentian Violet induced a small amount of SV40 DNA amplification which, in most cases, was dose related. The largest amount of amplification occurred at doses of 0.05 and 0.125 μg/mL. The authors suggested gene amplification may be involved in the

induction of tumors and has been mechanistically linked with the production of certain types of chromosome aberrations.[41]

## Carcinogenicity

*Gentian Violet.* The carcinogenicity of Gentian Violet was studied in 720 male and 720 female B6C3F1 mice.[42] Gentian Violet was dissolved in ethyl alcohol and sprayed directly into the feed at dose levels of 0, 100, 300, and 600 ppm. The ethyl alcohol was subsequently removed during a 30-minute blending process under vacuum. Both sexes of mice received all 4 doses for 12 months (120 male, 120 female), 18 months (120 male, 120 female), or 24 months (480 male, 480 female). Females ingested approximately 100, 250 to 275, and 500 mg of Gentian Violet per kilogram of body weight per week at each respective dose level, whereas males ingested approximately 75 to 100, 225 to 250, and 450 to 475 mg of Gentian Violet per kilogram of body weight per week at each dose level. There was no effect on feed consumption or body weight gain; however, a dose effect was noted for mortality rates. For controls, mortality of both sexes was less than 15% at 24 months but was approximately 28%, 27%, and 64% in females in the 100-, 300-, and 600-ppm dose groups, respectively. For males, mortality was 14%, 20%, and 23% in the 100-, 300-, and 600-ppm dose levels, respectively.

A positive dose response for hepatocellular carcinoma was noted in males at 24 months and in females at 18 and 24 months at the 300- to 600-ppm doses. There were statistically significant positive trends with respect to dose and (1) mortality due to liver neoplasms, (2) prevalence of liver neoplasms, and (3) time to onset of liver neoplasms in both males and females. In female mice, erythropoiesis in the spleen, atrophy of the ovaries, adenoma of the Harderian gland, and the presence of type A reticulum cell sarcomas in the urinary bladder, uterus, ovaries and vagina were seen. The authors concluded that Gentian Violet appeared to be a carcinogen in mice at several different sites.

The carcinogenicity of Gentian Violet was studied in 570 male and 570 female F344 rats.[42] Gentian Violet was dissolved in ethanol and sprayed directly into the feed at dose levels of 0, 100, 300, and 600 ppm. The ethanol was subsequently removed during a 30-minute blending process by vacuum. Both sexes of rats received all 4 doses for 12 months (60 male, 60 female), 18 months (60 male, 60 female), or 24 months (450 male, 450 female). Male and female rats fed 600 ppm Gentian Violet for 24 months showed a decrease in body weight. Average feed consumption was essentially equal in all groups. Mortality at the end of the study (24 months) was approximately 33% in the controls for both sexes and 66% in females of the high-dose group and 48 and 39% in males of the middle- and high-dose groups, respectively.

Following 24 months of dosing, there was a significant difference from the controls (1 case both sexes) in the incidence of follicular cell adenocarcinoma of the thyroid gland for both males (600 ppm; 5 cases) and females (300 and 600 ppm; 4 and 6 cases, respectively). Although the incidences were very low, there was a significant difference from the controls (1 and 0 cases for male and females, respectively) for hepatocellular adenomas in the middle- and high-dose groups of the males (3 and 4 cases, respectively) and the middle-dose group of the females (2 cases). A dose-time–related incidence of mononuclear cell leukemia was noted in the females.

## Clinical Assessment of Safety

### *Dermal Irritation/Sensitization*

Bielicky and Novak patch tested 11 patients with clinical signs of contact sensitivity to therapeutically used triphenylmethane dyes.[43] Seven dyes were used, including Basic Violet 3 and Gentian Violet. The patch tests were applied for 20 to 24 hours and were evaluated for 6 to 7 days. Because the skin was colored by the tested dyes, it was not possible to evaluate the erythematous reaction. Positive test reactions (itching ±; isolated papules +; edema, confluent papules, and infiltration ++; or vesicular reaction +++) were observed in 8 of the patients for Basic Violet 3. Positive test reactions were observed in 8 of the patients for Gentian Violet.

Gentian Violet is a weak sensitizer when used as an antiseptic therapeutic dye, except when used on ulcers and eczematized skin. Gentian Violet may produce not only allergic contact dermatitis but also necrosis in the interiginous areas.[44,45] The concentration of dyes for patch testing are 2% in water for Gentian Violet and 1% to 2% in water for Basic Violet 1.[46,47]

## Case Reports

### *Ocular Irritation*

*Gentian Violet.* Dhir et al[31] describe a case where a 60-year-old male had 1% Gentian Violet accidentally instilled into both his eyes by a medical practitioner. The patient complained of irritation, pain, and diminution of vision. On examination, his visual acuity was reduced to counting fingers ½ meter OD (right eye) and 6/36 OS (left eye). Moderate lid edema and blepharospasm were present. The conjunctivae were congested and chemosed. Corneas were hazy and edematous in both eyes. Biomicroscopic examination revealed punctate epithelial lesions scattered all over the cornea. The patient was treated with antibiotic, steroid, and cycloplegic drops and the eyes were patched. The corneal lesions healed in a period of 4 to 5 weeks leaving behind fine punctate opacities at the level of Bowman's membrane. At the end of 5 weeks, the visual acuity had improved to counting fingers at 1 meter OD and 6/9 OS. The marginal tear strip was absent. Tear breakup time was 5 seconds in both eyes. After 4 months, the tear secretion had not shown any improvement.

## Mucosal Irritation

Several case reports of 1% Gentian Violet used in infants mouths to treat thrush. Lesions, ulceration, blistering, and difficulty nursing were reported. Patients recovered after discontinuation of treatment.[48-52]

Piatt and Bergeson[53] described a case in which a 15-day old infant's tongue appeared white and fuzzy. The mother was advised to use 2% Gentian Violet as treatment. She applied the solution at least 10 to 12 times daily for 4 days. On the fifth day, she noted that the tongue looked unusual and the baby had become increasingly fussy and began to refuse to nurse. She then decreased the applications to twice daily and started formula feeding, but the child remained irritable and was subsequently brought to the emergency room. There, the patient was diagnosed as having severe candidiasis and macroglossia. The undersurface of the tongue, gingival grooves, and floor of the mouth were covered with large, purplish gray plaques that could be scraped off, leaving a bleeding surface. The lesions appeared similar to a caustic burn of the oral mucosa and tongue. When Gentian Violet was discontinued, the patient became much less irritable, fed well, and gained weight by the third day of hospitalization.

Quinby[1] described an epidemic of nosebleeds in apple packers who used packing trays dyed with Gentian Violet. The year before the epidemic, changes were made in sizing agents and chemical ingredients concerned with bonding the packing trays, which were made of recycled newsprint. Many of the packers complained that the trays were dustier than in earlier years. Loose tray dust was flushed into the faces of packers approximately 1000 times a day. Other employees who worked more than 10 feet from the packing operation had no nasal bleeding above that of the general population. When the plants stopped using newspaper trays, packers stopped bleeding. Conversely, packers started bleeding in plants that switched from virgin wood pulp to newspaper trays. Although trays made from virgin wood pulp contained similar blue dye, they did not cause bleeding. It was noted, however, that a few pulp workers in the plant that manufactured the virgin wood pulp trays also had nosebleeds only during the weeks in which they had added liquid or powdered blue dye to the batch.

## Dermal Effects

*Basic Violet 3.* Meurer and Konz[44] described a case in which a 2-year-old male developed a necrotic skin reaction in the gluteal fold after application of 2% Basic Violet 3 in aqueous solution to treat diaper dermatitis. After discontinuation of the solution, demarcation of the necrotic area and reepithelization of the wounded surfaces occurred.

*Gentian Violet.* Epstein[54] reported 3 cases in which patients developed sensitivities to Gentian Violet. In the first case, a 55-year-old female was being treated because of resistant stasis dermatitis of her right lower leg. After some time, Gentian Violet seemed to irritate. Patch tests with this drug (concentration not given) were negative. A 0.5-mL intradermal test of a 0.02% solution produced a positive delayed tuberculin–type reaction. In the second case, a 57-year-old female with eczema on her left ankle developed dermatitis when she used Gentian Violet as treatment. Patch tests with 1% Gentian Violet solution on normal skin were negative for 48 hours. After 72 hours, follicular lesions appeared that turned into a papulonodular, nonvesicular dermatitis. Intradermal tests with 0.02% Gentian Violet were positive. After 24 hours, a mild itching dermatitis with follicular swelling was present covering an area of 2 cm in diameter after 48 hours. In the third case, a 69-year-old male claimed that Gentian Violet had irritated him on previous occasions when used to treat lichen simplex on his ankles. Patch tests with a 1% alcoholic solution of Gentian Violet were negative; however, an intradermal test with 0.3 mL of a 0.001% Gentian Violet solution produced a papular tuberculin type reaction.

Lawrence and Smith[45] describe a case in which a benign ovarian dermoid was discovered in a 42-year old woman during a hospital stay for a leg ulcer. Following hysterectomy and bilateral oophorectomy, the wound became infected. Gentian Violet (concentration not given) was applied to the wound edge, and 10 days later an acute eczematous flare developed around the wound and leg ulcer. Patch tests for Gentian Violet (0.25% in water) resulted in + and ++ results for 48 and 96 hours, respectively, for the unoccluded test and + and + for 48 and 96 hours, respectively, for the occluded test.

## Bladder Irritation

*Gentian Violet.* Walsh and Walsh[55] reported the case of a 32-year-old female who mistakenly injected 1% Gentian Violet (in 2% alcohol) into her urethra instead of her vagina to treat severe pruritus. Within a few seconds, a burning pain in the lower abdomen developed, followed by urinary frequency and urgency and dysuria. Two days later, she noticed hematuria, which led to her admission to the hospital. An intravenous pyelogram suggested a mass lesion in the left side of the bladder. Cystoscopy showed gross inflammation and edema of the left side of the bladder with acute ulceration of the overlying mucosa and a large mass on the left side of the bladder. Her condition improved with a high intake of fluids, and a later cystogram showed a normal outline of the bladder with no evidence of the mass lesion. Histological examination of a slightly thickened area of the bladder showed extensive ulceration and nonspecific inflammatory changes with large numbers of eosinophils but no evidence of neoplasia. The urine was sterile.

## Hair Dye Epidemiology

Hair dyes may be broadly grouped into oxidative (permanent) and direct (semipermanent) hair dyes. The oxidative dyes consist of precursors mixed with developers to produce color, whereas direct hair dyes are a preformed color. Basic Violet 3, Basic Violet 1, and Basic Violet 4 are direct hair dye ingredients. Although the safety of individual hair dye ingredients is not addressed in epidemiology studies that seek to determine

links, if any, between hair dye use and disease, such studies do provide broad information. The CIR Expert Panel noted the conclusions of these reviews, including that personal use of hair colorants cannot be evaluated as to its carcinogenicity and that occupation as a hairdresser or barber entails exposures that are probably carcinogenic; that insufficient evidence exists to support a causal association between personal hair dye use and a variety of tumors and cancers such as acute leukemia, bladder cancer, multiple myeloma, and non-Hodgkin's lymphoma; and that the epidemiological evidence for personal use of hair colorants is inadequate and is not classifiable as to its carcinogenicity to humans.[56-58]

## Summary

Basic Violet 3, Basic Violet 1, and Basic Violet 4 are triphenylmethane direct (nonoxidative) hair dyes. The term *Gentian Violet* is used synonymously with *Basic Violet 1* and *Basic Violet* 3. These nonoxidative hair colorants have no reported uses.

Acute oral and intraperitoneal studies found an oral $LD_{50}$ greater than 2160 mg/kg in young rats and greater than 2600 mg/kg in adult rats and an intraperitoneal $LD_{50}$ of 64 mg/kg in young rats and 57 mg/kg in adult rats. The oral $LD_{50}$ of Basic Violet 3 was approximately 90 mg/kg and 650 mg/kg for young and adult rats, respectively. The intraperitoneal $LD_{50}$ was 17 mg/kg in both young and adult rats. Basic Violet 4 has an oral $LD_{50}$ of 549 mg/kg in male Sprague-Dawley rats and 308 mg/kg in females.

Basic Violet 1 was found to be strongly cytotoxic in a human fibroblast-like cell line. In the parotid salivary glands of miniature pigs, Basic Violet 1 caused swelling, followed by hardening and eventually shrinkage. Basic Violet 1 was mutagenic in *S typhimurium* strain TA1535 at low concentrations in the absence of S9 in one study but was found not to be mutagenic in 4 other strains of *S typhimurium* with and without S9. Concentrations of Basic Violet 1 in phage T4D higher than 200 μg/mL did not significantly increase mutation frequency, and doses higher than 91 μg/mL reduced phage yield.

Basic Violet 3 was found to be strongly cytotoxic in human fibroblast-like cells as well as in epithelial-like cells, although to a lesser extent.

In pregnant rats treated with Basic Violet 3 on gestational days 6 to 15, reduced maternal body weight was found at a dose of 10 mg/kg on gestational days 11 and 15; a decrease in maternal weight gain for the overall gestation period was also found. Maternal mortality was 9.4% in the 10-mg/kg dose. New Zealand white rabbits given 2 mg/kg or less Basic Violet 3 demonstrated decreased maternal body weight on gestational day 19 and a decreased maternal weight gain. Maternal mortality rates were 7.4% at 0.5 mg/mL, 15.4% at 1.0 mg/mL, and 22.6% at 2.0 mg/mL. In rats, the number of litters with malformed fetuses and the percentage of malformed fetuses per litter were significantly higher in the 10-mg/kg group. In New Zealand white rabbits, the average fetal body weight per litter was significantly lower than controls.

In CHO cells, chromosome damage ranged from 2.60 to 5.84 breaks per cell after exposure to Basic Violet 3 for 5 hours. Basic Violet 3 was mutagenic in strain TA1535 of *S typhimurium* only in the absence of S9. The genotoxicity of Basic Violet 3 was enhanced by irradiation with visible light in studies using *E coli*. An Ames test using *S typhimurium* strains TA98, TA100, and TA1537 demonstrated no mutagenic activity. However, in strain DG1669 of *S typhimurium*, Basic Violet 3 caused frameshift mutations.

In an oral carcinogenicity study of 100 to 600 ppm Gentian Violet in mice, there was a positive dose response for hepatocellular carcinoma in males at 24 months and in females at 18 and 24 months. There were also statistically significant positive trends linking dose with mortality due to liver neoplasms, prevalence of liver neoplasms, and time to onset of liver neoplasms in males and females. In female mice, adenoma of the Harderian gland and sarcomas of the urinary bladder, uterus, ovaries, and vagina were observed. Gentian Violet was classified as carcinogenic.

In a similar oral carcinogenicity study, Gentian Violet was administered in feed to male and female F344 rats at the same doses. When compared with controls after 24 months of dosing, a statistically significant increase in the incidence of follicular cell adenocarcinoma of the thyroid gland was reported for males and females. A statistically significant increase in the incidence of hepatocellular adenomas was reported for males dosed with 300 ppm and 600 ppm Gentian Violet. A dose-time–related incidence of mononuclear cell leukemia was also reported for females.

In rabbit eyes, Basic Violet 3 produced blepharitis with hyperemia, edema, and necrosis of the conjunctivae and nictating membrane, followed by an increasingly severe keratitis, then gross opacification, deformity, and vascularization of the cornea over the course of 3 weeks.

Application of 1% Gentian Violet to the conjunctival sac of rabbits led to conjunctival congestion and then necrosis of the conjunctiva. In *S typhimurium*, Gentian Violet was mutagenic in strain TA97 with and without S9 activation and in strain TA104 with S9. Gentian Violet was toxic but not mutagenic in an Ames assay, while causing reparable DNA damage in a Rosenkranz assay in CHO, in vitro in the presence of rat-liver S-9 fractions and chicken embryo cells and mouse bone marrow cells in vivo. Gentian Violet was highly toxic to chick embryos at a high dose. In CHO cells, Gentian Violet was found to be toxic. Gentian Violet caused a significant difference in the incidence of follicular cell adenocarcinoma of the thyroid gland of male and female rats as well as in the incidence of hepatocellular adenomas in male rats. Gentian Violet appeared to be a carcinogen in mice at several sites, including the liver, spleen, ovaries, Harderian gland, urinary bladder, and vagina.

Eight of 11 patients with sensitivity to triphenylmethane dyes had positive test reactions to exposure to Basic Violet 3. In a 2-year-old male, application of a 2% Basic Violet 3 in aqueous solution produced a necrotic skin reaction in the gluteal fold.

Apple packers experienced an epidemic of nosebleeds after exposure to packing trays dyed with Gentian Violet. Gentian Violet may act as a weak sensitizer when used as an antiseptic therapeutic dye and may produce contact dermatitis and necrosis in interiginous areas. Case reports showed that use of Gentian Violet to treat thrush in infants caused oral lesions and ulcerations that resolved following termination of treatment. The most recent comprehensive review of available epidemiology studies concluded that there is insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. A summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml.

## Discussion

The CIR Expert Panel recognized that these dyes are in limited use in cosmetics, if at all. Basic Violet 1, Basic Violet 3, and Basic Violet 4 are triphenylmethane dyes for which there is concern regarding genotoxicity and carcinogenicity. Gentian Violet, a term that is used synonymously with Basic Violet 1 and Basic Violet 3, was carcinogenic when fed to mice and rats, but the panel concluded that these data were of uncertain relevance based on the confusion regarding which of the 2 ingredients (Basic Violet 1 or Basic Violet 3) might have been used in the studies. The panel noted that Basic Violet 1, 3, and 4 contain quaternary ammonium ions and therefore the rate of penetration across the epidermis is expected to be slow, but because of the potential carcinogenic risk associated with these compounds, the panel considers that dermal absorption data, particularly as regard potential carcinogenicity, and a risk assessment are needed to complete this safety assessment.

In considering hair dye epidemiology data, the CIR Expert Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other end points, based on lack of strength of the associations and inconsistency of findings. Use of direct hair dyes, although not the focus in all investigations, appears to have little evidence of any association with adverse events as reported in epidemiology studies.

## Conclusion

The CIR Expert panel concluded that the data available on Basic Violet 1, Basic Violet 3, and Basic Violet 4 are insufficient to support the safety of these hair dye ingredients as used in cosmetic formulations.

### Authors' Note

Unpublished sources cited in this report are available from the Director, Cosmetic Ingredient Review, 1101 17th Street, Suite 412, Washington, DC 20036, USA.

### Declaration of Conflicting Interests

No potential conflict of interest relevant to this article was reported. F. Alan Andersen is employed by the Cosmetic Ingredient Review, and Catherine Diamante was employed by the Cosmetic Ingredient Review.

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