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Final Report on the Safety Assessment of Disperse Blue 1[1]

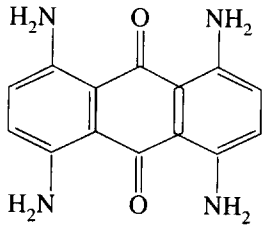
Abstract: The anthraquinone color Disperse Blue 1 is used in many nonoxidative hair dyes, colors and rinses. In vitro dermal penetration studies using skin from miniature pigs indicate this ingredient is poorly absorbed. All rats given Disperse Blue 1 orally at concentrations up to 3 g/kg survived. Reduced body weights and blue tissue samples were observed in short-term, subchronic, and chronic animal studies. No skin irritation was observed with concentrations up to 10%, but Disperse Blue 1 was a moderate sensitizer in guinea pigs. Disperse Blue 1 was mutagenic in several test systems. In feeding studies, the ingredient produced a significant increase in urinary bladder neoplasms in male and female rats. Equivocal results were reported in studies with male mice and negative results were reported for female mice. A dermal carcinogenesis study in mice was negative. Further evaluation of the carcinogenesis data suggests that the urinary bladder neoplasms appear to be associated with bladder calculi rather than arising from a genotoxic mechanism. Such bladder calculi do not appear to form in humans. Based upon these data and the facts that dermal exposure produced no evidence of carcinogenesis, that the ingredient is poorly absorbed, and that exposure to hair dyes is brief, it was concluded that Disperse Blue 1 is safe for use in hair dyes at concentrations up to 1%. **Key Words:** Disperse Blue 1—Dermal penetration studies—Guinea pig—Mouse—Carcinogenesis.

Disperse Blue 1 is an anthraquinone color used in nonoxidative hair dyes, colors, and rinses. The following report is a summary of the safety data on this ingredient.

CHEMISTRY

Definition and Structure

Disperse Blue 1 (CAS No. 2475-45-8) is classed chemically as an anthraquinone color. It conforms to the formula (Nikitakis et al., 1991):



[1] Reviewed by the Cosmetic Ingredient Review Expert Panel.

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Other names for Disperse Blue 1 are: 9,10-Anthracenedione, 1,4,5,8-Tetraamino-; 1,4,5,8-Tetraamino-9,10-Anthracenedione; CI 64500 (Nikitakis et al., 1991); 1,4,5,8-Tetraaminoanthraquinone (NTP, 1986); CI Disperse Blue 1; and CI Solvent Blue 18 (IARC, 1990).

Chemical and Physical Properties

Disperse Blue 1 is a blue-black microcrystalline powder (NTP, 1986). It has a molecular weight of 268.28 (Aldrich, 1992) and a melting point of 332°C (NTP, 1986). It is soluble in water (Kuroiwa and Ogasawara, 1973), acetone, ethanol, and cellosolve, and slightly soluble in benzene and linseed oil (NTIS, 1981).

Method of Manufacture

Disperse Blue 1 may be prepared by acylation of 1,5-diaminoanthraquinone with oxalic acid, then nitration in sulfuric acid, followed by hydrolysis and reduction to the tetraamino compound. Another method for production is by the reduction of mixed 1,5- and 1,8-dinitroanthraquinone to the corresponding diamino compounds, followed by acetylation, nitration, reduction, and hydrolysis (Society of Dyers and Colourists, 1971).

Commercial preparations of Disperse Blue 1 are approximately 50% Disperse Blue 1, 30% structurally related compounds, and 20% water. These preparations contain approximately equal amounts of dyestuff and lignosulfonate dispersants (Burnett and Squire, 1986; NTP, 1986).

One U.S. company markets Disperse Blue 1 with a dye content of ~30% (Aldrich, 1992).

Impurities

Using mass spectrometry, two major impurities in Disperse Blue 1 were identified: an isomer of Disperse Blue 1 and triaminonitroanthraquinone (NTP, 1986). Using high-performance liquid chromatography, the concentrations of these impurities were approximately 25% for the isomer and 6% for triaminonitroanthraquinone. No nitrosamine impurities were identified in the material tested.

Analytical Methods

Disperse Blue 1 may be determined at concentrations as low as 0.1–0.5 mg/ml in environmental and biological samples using a polarographic method (Popescu and Barbacaru, 1985). Spectrophotrometric determination has been used to determine Disperse Blue 1 sorbed on polyethylene terephthalate fibers by dye extraction in mixed solvent systems (Madan and Khan, 1978).

Ultraviolet/Visible Data

Tables 1 and 2 list spectral data obtained from analyzing Disperse Blue 1 in different solvents with a Cary 188 (NTP, 1986).

USE

Cosmetic

In general, disperse dyes are used in temporary hair color preparations in the form of rinses and colored setting lotions as well as in semipermanent hair dye preparations (Wilkinson and Moore, 1982).

Disperse Blue 1 is used as a hair colorant in hair dyes, colors, and rinses (Nikitakis, 1988). The product formulation data submitted to the Food and Drug Administration (FDA) in 1994 reported that it was used in 112 hair dyes and colors, and in two formulations classified as "Other Hair Coloring Preparations" (Table 3) (FDA, 1994). Concentration of use values are no longer reported to the FDA by the cosmetic industry (Federal Register, 1992). However, it was reported to the Cosmetic, Toiletry, and Fragrance Association that Disperse Blue 1 is used at up to 0.62% in semipermanent hair dyes and is not used in conjunction with hydrogen peroxide (Clairol, 1994).

Hair coloring formulations are applied to or may come in contact with hair, skin (particularly at the scalp), eyes, and fingernails. Individuals dyeing their hair may use such formulations once every few weeks, whereas hairdressers may come in contact with products containing these ingredients several times a day. Under normal conditions of use, skin contact with hair dye is restricted to 30 min.

The hair dyes containing Disperse Blue 1, as coal tar hair dye products, are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The following caution statement should be displayed conspicuously on the labels of coal tar hair dyes:

> **Caution:** This product contains ingredients that 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 eyelashes or eyebrows; to do so may cause blindness.

At its February 11, 1992, meeting, the CIR Expert Panel issued the following policy statement on coal tar hair dye product labeling:

> The Cosmetic Ingredient Review (CIR) Expert Panel has reviewed the cosmetic industry's current coal tar hair dye product labeling, which recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 24 hours after application of the test material and prior to the use of a hair dye formulation.

TABLE 1. *Absorption maxima in the UV for Disperse Blue 1 in methanol*

λ_{max} (nm)	ϵ
237	18,010 ± 690 (δ)
271	6,540 ± 44 (δ)
320 (shoulder)	2,061 ± 29 (δ)
594	5,957 ± 93 (δ)

TABLE 2. *Absorption maxima in the UV for Disperse Blue 1 in acetonitrile*

λ_{max} (nm)	ϵ
237	33,616 ± 646 (δ)
271	12,564 ± 363 (δ)
319 (shoulder)	4,940 ± 288 (δ)
323 (shoulder)	3,759 ± 252 (δ)

Since the recommendation on the industry's adopted labeling establishes a procedure for individual user safety testing, it is most important that the recommended procedure be consistent with current medical practice.

There is a general consensus among dermatologists that screening patients for sensitization (allergic contact dermatitis) should be conducted by the procedures used by the North American Contact Dermatitis Group and the International Contact Dermatitis Group (North American Contact Dermatitis Group, 1980; Eierman et al., 1982; Adams et al., 1985). These procedures state that the test material should be applied at an acceptable concentration to the patient, covered with an appropriate occlusive patch, and evaluated for sensitization 48 and 72 hours after application. The CIR Expert Panel has cited the results of studies conducted by both the North American Contact Dermatitis Group and the International Contact Dermatitis Group in its safety evaluation reports on cosmetic ingredients (Elder, 1985).

During the August 26–27, 1991, public meeting of the CIR Expert Panel, all members agreed that the cosmetic industry should change its recommendation for the evaluation of the open patch test from 24 hours to 48 hours after application of the test material.

The industry was advised of this recommendation and asked to provide any compelling reasons why this recommendation should not be made by the Expert Panel and adopted by the cosmetic industry. No opposition to this recommendation was received. At the February 11, 1992, public meeting of the CIR Expert Panel, this policy statement was adopted.

Noncosmetic

Disperse Blue 1 is used to dye nylon, cellulose acetate and triacetate, polyester, and acrylate fibers. It has also been used in surface dyeing of thermoplastics, and as a solvent dye in cellulose acetate plastics (NTIS, 1981).

BIOLOGY

Dermal Penetration

The dermal penetration of a formulation containing 1% Disperse Blue 1 was tested in vitro. Skin sections (~1 inch in diameter) from miniature pigs were

TABLE 3. *Cosmetic product formulation data on Disperse Blue 1 (FDA, 1994)*

Product category	Total no. of formulations in category	Total no. of formulations containing ingredient
Hair dyes and colors	1,458	110
Other hair coloring preparations	73	2
1994 Totals		112

prepared and mounted on Franz diffusion cells and 100 μl of the formulation (≈1.5 mg Disperse Blue 1 per cm^2) was applied to the epidermal surface. Three skin samples each were treated for either 30 min or 3 h, followed by rinsing. Two skin sections received no treatment and were used as control samples. The fluid in the receptor chambers was removed for analysis at 24-h intervals until 72 h post-treatment.

Overall, dermal penetration of Disperse Blue 1 did not exceed 0.15% of the applied dose, even after 3 h of exposure. The amount of Disperse Blue 1 that penetrated the skin samples was greater for the samples treated for 3 h than those treated for 30 min. The only sample for which Disperse Blue 1 was not detected was one that was treated for 30 min. Between 42 and 61% of the applied dose was collected at the first 24 h interval, and smaller amounts were obtained in successive collections (Clairol, 1991).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Groups of five male and five female F344/N rats were administered 188, 375, 750, 1,500, and 3,000 mg/kg commercial grade Disperse Blue 1 (without lignosulfonate dispersants) in corn oil by gavage. The animals were observed twice a day and necropsy was performed on the animals when they were killed after 14 days. All of the animals survived the study. No final mean body weights were recorded. The only clinical observation was blue colored urine from day 1 through days 2–6 (NTP, 1986).

Using the same protocol, groups of five male and five female $B6C3F_1$ mice were also tested. The dosages administered were 125, 250, 500, 1,000, and 2,000 mg/kg Disperse Blue 1 (without lignosulfonate dispersants) in corn oil. All of the mice survived the study and blue urine was observed from day 2 through days 3–6 (NTP, 1986).

Short-Term Oral Toxicity

Disperse Blue 1 (minus lignosulfonate dispersants), at concentrations of 3,100, 6,200, 12,500, 25,000, or 50,000 ppm, was administered in the feed to groups of five male and five female F344/N rats and $B6C3F_1$ mice for 14 consecutive days. Control groups of animals were given untreated feed. Necropsy was performed on the animals at the time of death or when they were killed on day 16. The following deaths occurred during the study: two female rats fed the 50,000 ppm diet, the mice fed the 25,000 and 50,000 ppm diets, and three male and two female mice fed the 12,500 ppm diet. Notable reductions in weight gain was observed in both the rats and mice fed the 6,200 ppm diet, and in the rats fed 12,500 ppm Disperse Blue 1. Feed consumption was not measured. Treatment-related clinical signs were observed only in the rats of the high-dosage group and included inactivity, hunched back, and sunken eyes. The mice fed the diets of 12,500 ppm Disperse Blue 1 and greater were inactive. All of the animals had blue urine, and, at necropsy, most of the organs of all of the animals were blue (NTP, 1986).

In another study, 1 g/kg Disperse Blue 1 (containing 50% lignosulfonate dispersants) was administered daily to groups of three male and three female Fischer 344 rats by gavage for 1, 2, or 3 consecutive days. Another group of animals was fed a diet containing 1.0% Disperse Blue 1 for 4 days. All of the animals were killed on the day following the last administration. Necropsy was performed on all of the animals, and the urinary bladder and kidneys were examined microscopically. In the rats receiving Disperse Blue 1 by gavage, the dye accumulated in the kidney tubules and caused hyperplasia of the renal pelvis epithelium. Nephropathy was observed in most of the rats and was even more severe than that seen in the early stages of the chronic disease in older rats. The urinary bladder was normal in these animals. In the rats receiving Disperse Blue 1 in the diet, no dye accumulated in the kidneys after 4 days, but there was low grade hyperplasia of the bladder urothelium with varying amounts of epithelial erosion and submucosal edema in all of the animals. Clumps of the dye were found adhered to eroded areas. Similar effects were observed in the animals fed the diet containing Disperse Blue 1 without dispersants. The investigators concluded that the dispersants had no major effect on the absorption or elimination of Disperse Blue 1 (Burnett and Squire, 1986).

This same protocol was repeated with the following changes in dosages: each daily gavage dose was split into two doses of 500 mg/kg; 0.5% Disperse Blue 1, and 0.25 and 0.5% of Disperse Blue 1 without dispersants were tested via the diet. The results of these studies were comparable to those seen in the first study (Burnett and Squire, 1986).

Subchronic Oral Toxicity

Groups of 10 F344/N rats of each sex were fed diets containing 1,200, 2,500, 5,000, 10,000, or 20,000 ppm Disperse Blue 1, and groups of 10 $B6C3F_1$, mice of each sex were fed diets containing 600, 1,200, 2,500, 5,000, and 10,000 ppm Disperse Blue 1 for 13 weeks. A commercial grade of Disperse Blue 1 (without lignosulfonate dispersants) was used. Control groups of animals were fed untreated feed. Feed consumption was monitored and the animals were weighed weekly and were observed regularly for signs of toxicity. Necropsy was performed on rats dying during the study and those killed at the termination of the study.

Two male rats fed the diet containing 10,000 ppm Disperse Blue 1 died during the study. These deaths were not considered compound related because no significant compound-related pathologic effects were found, and because all of the rats from the 20,000 ppm treatment group survived. The final mean body weights of the male rats from the 10,000 and 20,000 ppm treatment groups were 8 and 14% lower, respectively, than that of the controls; and female rats from the 20,000 ppm treatment group had 10% lower body weights. Feed consumption was comparable between the treated groups and the control groups. The urine of the treated animals was blue. The extrahepatic bile ducts of four male rats fed the 20,000 ppm diet were distended by aggregates of dark-blue crystalline material. The following lesions were considered compound related in both male and female rats fed diets

containing 2,500 ppm Disperse Blue 1 or greater: pigmentation of the thyroid gland follicle, renal pigmentation and/or dilation, nephrosis, chronic inflammation and/or hyperplasia of the transitional epithelium of the urinary bladder, and calculi of the urinary tract. No compound-related lesions were found in the rats fed 1,200 ppm Disperse Blue 1.

In the study using mice, seven male and four female mice from the 10,000 ppm group, one male from the 5,000 ppm group, and two males from the 1,200 ppm group died during the study. The final mean body weights of the male mice fed 10,000 ppm Disperse Blue 1 were 34% lower than that of the controls. Female mice from the 5,000 ppm and 10,000 ppm groups had 7 and 17% lower body weights, respectively, than their controls. The following compound-related lesions were observed in both male and female mice fed diets of 2,500 ppm and greater: chronic inflammation and/or hyperplasia of the transitional epithelium of the urinary bladder, pigmentation of the thyroid gland follicles, renal pigmentation, nephrosis, pigment calculi of the urinary tract, and focal myocardial necrosis. Male rats also had mild degeneration of the germinal epithelium of the testis (NTP, 1986).

Chronic Oral Toxicity

A dye and base composite containing 1.54% Disperse Blue 1 was evaluated in a 2-year chronic oral toxicity test. Groups of six male and six female beagle dogs were fed 19.5 and 97.5 mg/kg per day of the composite in their diet. A control group of dogs was fed untreated feed. The animals were observed daily for signs of toxicity. Body weight, feed consumption, ECG, blood pressure, pulse rate and body temperature determinations, and funduscopic examinations were conducted initially and at 3, 6, 12, 18, and 24 months. Hematologic, blood chemical, and urinalysis parameters were determined at the same times for all high-dosage and control dogs, as well as three male and three female dogs of the low-dosage group. Necropsy was performed on one dog of each sex from each group at 6, 12, and 18 months and on all survivors at 24 months. Tissue samples were examined microscopically. Additionally, sections of liver and urinary bladder were examined by electron microscopy.

No significant differences between control and test groups were seen in any of the parameters studied. All of the dogs in both test groups excreted blue-brown urine; however, urine analyses were negative for renal toxicity. The investigators found that urine collected after overnight fasting was normal in color, suggesting probable rapid clearance of the dye (Wernick et al., 1975).

Dermal Irritation and Sensitization

An emulsion composed of 15 mg Disperse Blue 1 dissolved in 4 ml Freund's Complete Adjuvant (FCA) and emulsified with 4 ml physiologic saline was tested for sensitization potential using female albino, Pirbright guinea pigs. Groups of 10 guinea pigs were given six intradermal injections of 0.1–0.15 ml of the emulsion. The injections were made in a semicircular arc on the clipped and shaved shoulder area, and were administered in such a way that all of the emulsion was used for

the 10 animals. These injections were repeated on days 5 and 9, leaving a gap of 2–3 cm between the rows of injections. Each animal received a total of ~4.5 mg during this induction period. After an 11-day non-treatment period, 0.05 ml of 1% Disperse Blue 1 dissolved in acetone was applied to the right clipped and shaved flank of the guinea pigs. Reactions were scored on a scale of 0–3 (3 being the most severe reaction) after 24, 48, and 72 h. Disperse Blue 1 was classified as a moderate sensitizer, having a mean response of 1.30 at 24 h, 1.75 at 48 h, and 2.00 at 72 h (Hausen and Sawall, 1989).

The irritant threshold of Disperse Blue 1 was also measured. Ten guinea pigs were treated with FCA and physiologic saline following the same injection procedures as the sensitization study. Disperse Blue 1, at concentrations of 1, 3, and 10%, were then applied to one flank of each animal, and the reactions were read after 24 h. No positive responses were observed. The authors concluded that the irritation threshold for Disperse Blue 1 was greater than 10% (Hausen and Sawall, 1989).

Reproduction and Developmental Toxicity

A composite of dyes and base components found in commercial semipermanent hair coloring products was administered in the diet to rats to determine fertility and reproductive effects. The composite containing 1.54% Disperse Blue 1 was incorporated into the diet at concentrations of 0.195 and 0.78% composite dye. The study was conducted in two parts using a total of six groups of 10 male and 20 female Sprague–Dawley CD rats each. In Part I, females were fed a basal diet and the males were fed the test diets for 8 weeks prior to and during the mating period. This was reversed in Part II; the males were fed the basal diet and the females were fed the test diets for 8 weeks prior to mating, during gestation, and for 21 days of lactation. Two control groups of animals were fed untreated feed. One pregnant female from each group was killed on day 13 of pregnancy. The uterus was examined an the fetuses were inspected for abnormalities. The remaining females were allowed to deliver normally.

Male and female fertility was not impaired. No significant effects were observed in length of gestation, number of females with resorption sites, live pups per litter, pup body weight, and pup survival. The female fertility index and average pup weight in the high-dosage groups in Parts I and II, respectively, were lower than the control values, but were not statistically significant. Feed consumption and body weight gains were not affected. No abnormalities were found in fetuses after 13 days of gestation or among offspring grossly examined at 21 days of age (Wernick et al., 1975).

This dye composite containing 1.54% Disperse Blue 1 was also tested for teratogenic effects. Three groups of 20 male and 20 female CFE-S (Carworth Farms) rats were mated one male to one female. The females were then fed diets containing either 1,950 or 7,800 ppm of the composite on days 6–15 of gestation. A control group of females was also mated but given untreated feed. The females were killed on day 19 of gestation and were examined for the number and distribution of fetuses, the number of corpora lutea, live and stillborn fetuses, and early

and late resorptions. After the fetuses were weighed, measured, and examined for gross abnormalities, one third of them were examined for visceral anomalies and the remainder were evaluated for skeletal malformations. No increase in either reproductive or fetal anomalies was found (Wernick et al., 1975).

The dye composite also was evaluated for teratologic effects using New Zealand white rabbits that were artificially inseminated. Four groups of 12 rabbits each were dosed daily by gavage on days 6–18 of gestation with either 19.5 or 97.5 mg/kg composite, 97.5 mg/kg composite without dyes, or 1 ml/kg of the vehicle (0.5% aqueous methylcellulose). All of the rabbits were killed on day 30 of gestation and examined for pregnancy, average fetal weights, maternal weight gain, numbers of corpora lutea, implantations, resorptions, and live and stillborn fetuses. One half of the fetuses were killed and examined for visceral and skeletal abnormalities and the other half was incubated for 24 h to evaluate viability and were then killed and examined. No evidence of teratogenicity was found (Wernick et al., 1975).

MUTAGENICITY

The mutagenic potential of Disperse Blue 1 was evaluated in the Ames test using *Salmonella typhimurium* strains TA1535, TA100, TA98, and TA97. Disperse Blue 1 was tested at concentrations ranging from 10 to 2,000 μg/plate for strains TA1535, TA100, and TA98, and from 0.1 to 100.0 μg/plate for strain TA97. Tests were conducted in triplicate both with and without metabolic activation with S9 fractions from Aroclor 1254–induced male Sprague–Dawley rats and male Syrian hamsters. Solvent and positive controls were run concurrently with each trial. The positive controls used for the protocols without metabolic activation were: sodium azide for strains TA100 and TA1535, 4-nitro-*o*-phenylenediamine for TA98, and 9-aminoacridine for TA97. In the protocols with metabolic activation, 2-aminoanthracene was used as the positive control. The experiment was performed twice. Disperse Blue 1 was mutagenic in strains TA98 and TA97 both with and without metabolic activation, and in strain TA1535 only in the presence of S9. Disperse Blue 1 was not mutagenic in strain TA100 (Zeiger et al., 1988).

In another Ames test, Disperse Blue 1 was tested at concentrations of 100, 500, and 2,000 μg/plate using *S. typhimurium* strains TA1538, TA1537, TA1535, TA100, and TA98. 2-Anthramine was used as the positive control for all five strains tested with metabolic activation. The positive controls used in the tests without metabolic activation were 4-nitroquinoline *N*-oxide for strains TA1538 and TA98, methyl methanesulfonate for TA100, ethyl methanesulfonate for TA1535, and 4-aminoacridine for TA1537. Equivocal results were obtained in tests with TA1537 both with and without metabolic activation. Disperse Blue 1 was not mutagenic in strains TA1538, TA1535, TA100, and TA98 with or without activation (Brown and Brown, 1976).

Disperse Blue 1 was also tested for mutagenic potential in the L5178Y mouse lymphoma cell mutation assay. Two trials were run. In the first trial, concentrations ranging from 10 to 160 μg/ml Disperse Blue 1 were tested and, in the second trial, concentrations of 2.5–80 μg/ml were used. Both trials were conducted with-

out S9 activation. The cells were exposed to Disperse Blue 1 for 4 h, followed by washing, suspension in medium, and incubation for 48 h. The cells were then plated with medium containing trifluorothymidine to select for cells resistant to this compound. Disperse Blue 1 was positive for mutagenicity in both trials (Myhr et al., 1990).

Positive responses were also obtained when Disperse Blue 1 was tested in the chromosome aberration test. Chinese hamster ovary (CHO) cells were incubated with Disperse Blue 1 alone and with the dye and S9 mixture. The cells were then exposed to Colcemid. Two trials were conducted without S9 activation using concentrations ranging from 8 to 10 μg/ml Disperse Blue 1. Two additional tests were conducted with S9 activation using concentrations of 5–10 μg/ml Disperse Blue 1 in one trial, and using 8–10 μg/ml in the other. Disperse Blue 1 caused positive responses in tests with metabolic activation at concentrations of 7.5 μg/ml and greater, and without metabolic activation at concentrations of 9 μg/ml and greater (Anderson et al., 1990).

In sister chromatid exchange tests, CHO cells were incubated with Disperse Blue 1 at concentrations ranging from 0.33–10 μg/ml both with and without metabolic activation. Colcemid was added to the cells and slides were prepared for evaluation. Disperse Blue 1 evoked a positive response in tests without metabolic activation at concentrations of 3.3 μg/ml and greater, and evoked a weak positive response in tests with S9 activation at a concentration of 10 μg/ml (Anderson et al., 1990).

A semipermanent hair dye containing 0.12% Disperse Blue 1 was tested in a heritable translocation study. The hair dye was topically applied to 25 male outbred Sprague–Dawley Charles River CD rats twice a week for 10 weeks. A control group of 25 male rats received no applications. Each of the males from both the experimental and control groups (F_0 generations) were then mated to one female each week for 3 weeks. The females were individually housed and allowed to deliver naturally. Twelve weeks after birth, 100 male offspring (F_1 generation) were each mated to one female rat each week for 3 weeks. The females were killed on days 14–16 of gestation, and the number of live and dead fetuses, implantations, and resorptions were recorded.

The frequent application of the hair dye had no significant effect on the body weight gains of the F_0 males. A range of 88–96% fertility (number of pregnancies versus the number of matings) was observed for all three matings of the F_0 males. No effects on fertility or reproduction were observed during three matings of the 100 F_1 males either (Burnett et al., 1981).

CARCINOGENICITY

A commercial grade of Disperse Blue 1 (minus lignosulfonate dispersants) was tested for carcinogenic potential by NTP (1986). Groups of 50 male and 50 female F344/N rats were fed diets containing 1,250, 2,500, or 5,000 ppm Disperse Blue 1, and groups of 50 male and 50 female B6C3F_1 mice were fed diets containing 600, 1,200, or 2,500 ppm Disperse Blue 1 for 2 years. Control groups of animals were fed an untreated diet. The animals were observed twice a day, and clinical signs

were recorded once per week. Body weights were monitored weekly for the first 13 weeks and monthly thereafter. Necropsy was performed on all of the animals either at the time of death or when they were killed at the end of the study.

The daily consumption of Disperse Blue 1 for the low, mid, and high dosage rats were: 45, 95, and 217 mg/kg, respectively, for the males, and 56, 111, and 240 mg/kg, respectively, for the females. Throughout the study, the mean body weights of the male and female rats in the high dosage group were lower than that of the control groups, and the rats from the mid dosage group had marginally lower body weights than the controls. The survival rates of the high dosage male and female rats were significantly reduced after 65 and 72 weeks, respectively. The males from the mid dosage group had marginally reduced survival rates, until week 100 when the rate became significantly reduced. Clinical signs of toxicity observed during the study included blue urine, firmness in the area of the urinary bladder, wet fur in the pelvic area, blue fur and extremities, and feces stained blue-green. Some of the female rats fed the high dosage had blue crusty material in the vaginal area.

The most significant pathologic changes were found in the urinary system of both the male and female rats. There was an increased incidence of the following nonneoplastic lesions: renal and urinary bladder calculi, renal casts, hydroephrosis and renal degeneration, renal and urinary bladder epithelial hyperplasia, urinary bladder squamous metaplasia, and pigmentation of the kidneys and urinary bladder. A number of different tumor types were also found in the urinary bladder. There was a dosage-related incidence of the following neoplasms: transitional cell neoplasms and leiomyosarcomas in the male rats of the mid and high dosage groups; squamous cell neoplasms in the males of the high dosage group; transitional cell papillomas and transitional cell carcinomas in the females of the mid and high dosage groups; and squamous cell papillomas, squamous cell carcinomas, leiomyoma, and leiomyosarcoma in the females fed the high dosage. Lipomatosis of the urinary bladder was also found in nine mid dosage female rats and one high dosage female rat; but it could not be determined if these lesions were neoplastic.

The investigators noted that urinary bladder calculi were often found in the rats with bladder neoplasms, which suggested that the calculi may have influenced the occurrence of the neoplasms in the rats. However, a number of rats had urinary bladder neoplasms without observed calculi. There was also a marginally increased incidence of pancreatic islet cell adenomas or carcinomas (combined) in male rats treated with Disperse Blue 1. Based upon these observations, the conclusions were that there was "clear evidence of carcinogenicity for male and female F344/N rats as shown by the increased occurrence of transitional cell papillomas and carcinomas, of leiomyomas and leiomyosarcomas, and of squamous cell papillomas and carcinomas of the urinary bladder."

In the study with the $B6C3F_1$ mice, the daily consumption of Disperse Blue 1 for the low, mid, and high dosage animals was calculated to be 112, 239, and 540 mg/kg per day for the males, and 108, 235, and 520 mg/kg per day for the females. The mean body weight of the females fed the high dosage were generally lower than that of the female controls, while the females of the low dosage group had

generally greater body weights. The mean body weights of the male mice were comparable to that of the controls. With the male mice, there was a significant trend toward lower survival with increasing doses of Disperse Blue 1; however, none of the dosage groups had a significant reduction in survival in pairwise comparisons with controls. There was no significant difference in survival between the groups of female mice and the controls. Treatment-related clinical signs of toxicity in the male rats included alopecia, externally cannibalized genitalia, and scratches. Both male and female mice had blue fur, blue urine, and firmness in the area of the urinary bladder.

Non-neoplastic lesions found at significantly increased incidences in the urinary system of both the male and female mice were blue pigmentation of the urinary bladder and kidneys, inflammation and epithelial hyperplasia in the urinary bladder, calculi in the urinary bladder lumen, fibrosis of the urinary bladder, casts in the renal tubular lumina, and renal tubular degeneration. There were no significant increases in the number of neoplastic lesions found in the urinary bladder or kidneys. Neoplastic lesions found at increased incidences elsewhere in the body were hepatocellular adenomas in the females of the low dosage group, hepatocellular adenomas or carcinomas (combined) in the low and mid dosage male mice, and alveolar/bronchiolar adenomas or carcinomas (combined) in the high dosage male mice. Based on these findings, the authors concluded that there was "equivocal evidence of carcinogenicity of [Disperse Blue 1] in male $B6C3F_1$", and that there was "no evidence of carcinogenicity of [Disperse Blue 1] in female $B6C3F_1$ mice".

Burnett and Squire (1986) conducted chronic studies to determine the effect of dietary administration of Disperse Blue 1 on the urinary system of Fischer 344 rats. Groups of 20 male and 20 female rats were fed a diet containing 0.01 or 0.10% Disperse Blue 1 for 19 months. Another group of 60 male and 60 female rats were administered a diet of 1.0% Disperse Blue 1 for 6 months. A control group of 40 male and 40 female rats were fed untreated diet. Body weights and feed consumption were recorded weekly for the first 3 months, and then monthly thereafter. These parameters were reduced only in the 1.0% Disperse Blue 1 group, and were rarely <90% of the controls. Urine samples were collected after 4, 8, 18, 23, and 35 weeks for pH determination. A few statistically significant different pH values were found, but the incidence of these changes were scattered. The urine of all the treated animals was blue.

After 5, 9, and 17 weeks on the diets, 3–5 rats were killed from each of the groups for necropsy. Multiple small dark blue particles and sediment were found in many of the urinary bladders from the rats fed the 1.0% Disperse Blue 1 diet. These particles increased in size with time. With one exception, all of the rats with tumors had one or more large calculi. The calculi ranged in weight from 0.013 to 0.402 g in male rats, were smooth and very dark blue, and consisted mainly of Disperse Blue 1. The calculi found in the female rats ranged in weight from 0.687 to 6.170 g, were generally larger than those found in the male rats, were rough, blue-brown and occasionally mottled in appearance, and were composed of mostly calcium phosphate.

The results of autoradiographic examination of the urinary bladder were exten-

sive labeling in the transitional epithelial cell nuclei of the rats fed the 1% Disperse Blue 1 diet. The extent of labeling was correlated with the extent of simple or papillary hyperplasia. Hyperplasia was slight to moderate at week 5, and metaplasia was present at weeks 9 and 17. Several of the animals also had clumps of dye present on the epithelium. One male rat had a transitional cell papilloma and one female rat had a squamous cell papilloma. No increase in nuclear labeling and no lesions were found in the bladders of the animals from the 0.01 and 0.1% Disperse Blue 1 treatment groups.

Changes in the kidneys were observed only in the animals fed the 1.0% Disperse Blue 1 diet for 17 weeks. The severity of these changes correlated with the amount of dye present in the renal tissues and the length of treatment. Tubular degeneration and regeneration with interstitial fibrosis and inflammation were the most common lesion observed. The tubular detail was often distorted by dye present within the tubules. Hyperplasia of pelvis epithelium and dye particles in or on the pelvis epithelium were also observed. No dye or treatment-related changes were found in the kidneys from the rats fed the 0.01 and 0.1% Disperse Blue 1 diets for as long as 9 weeks.

After 6 months, 10 male and 10 female rats fed the 1.0% Disperse Blue 1 diet and 12 male and 12 female rats fed the control diet were killed and their urinary bladders and kidneys were examined. The urinary bladders of most of the male rats contained calculi weighing up to 400 mg. Fewer calculi were found in the urinary bladders of the female rats, but fine dark blue sediment was often present. Moderate epithelial hyperplasia of the bladder was found in all of the rats, and squamous metaplasia was present in most of the animals. Other changes included dye on or beneath the epithelium, two rats with squamous cell papilloma, and one rat with transitional cell carcinoma. There was also focal accumulation of histiocytes beneath the bladder epithelium in association with dye particles in two of the rats. In the kidneys, Disperse Blue 1 was found in the tubules of all of the rats and pelvis epithelial hyperplasia was present. Some of the animals also had squamous metaplasia of the pelvis epithelium.

The remaining animals from the 1.0% Disperse Blue 1 group were fed the control diet for an additional 13 months and the calculi from the urinary bladders of 15 male and 15 female rats were surgically removed after 2 weeks on the control diet. The object of this procedure was to determine whether, without further exposure to the dye, the persisting foreign material could influence carcinogenesis. The number of bladder calculi and the development of severe bladder lesions were much greater in the female rats that had surgery than those that did not. The authors concluded that surgery stimulated the formation of even more calculi in the female rats. In the male rats, calculus development was minimal in those that underwent surgery and the degree of hyperplasia–metaplasia was decreased. In the males not receiving surgery, one transitional cell papilloma was found and most of the animals had minimal hyperplasia.

The urinary bladder and kidneys of the animals maintained continuously on the 0.01 and 0.1% Disperse Blue 1 diets for 19 months were comparable to those of the control animals.

Two nonoxidative hair dye formulations containing 0.10 and 0.3% Disperse

Blue 1 were tested for carcinogenic potential using Swiss Webster mice. Each formulation was applied to the clipped interscapular region of 60 male and 60 female mice three times a week for 20 months. Two control groups had the same shaving schedule but were left untreated. The mice were observed regularly for signs of toxicity. Ten mice of each sex were killed from each group after 9 months for clinical tests, hematology profiles, and necropsy. Necropsy was performed on the remaining animals either at the time of interim death or when they were killed at the end of the study.

No adverse effects on body weight gains or survival were observed in either of the treatment groups, and there was no evidence of toxicity from the hematologic or urinary values. Some of the animals had chronic inflammation of the skin, but this was also observed in the control animals. Hemangiomas of the liver, adenomas of the lungs, and malignant lymphomas were observed in some of the treated animals, but the incidences of these neoplasms were not significantly different from those observed in the two control groups. The authors noted that these lesions are commonly found in Swiss Webster mice and concluded that no carcinogenic effects were clearly indicated (Jacobs et al., 1984).

Risk Assessments

Reviewing the data of the NTP (1986) and Burnett and Squire (1986) studies, Couture-Haws et al. (1994) cited the weight of the evidence for a secondary carcinogenic mechanism of action for this ingredient. Specifically, they believed that Disperse Blue 1 acted through a threshold mechanism involving the formation of urinary calculi to induce bladder tumors in rats. They noted that not only was the occurrence of bladder neoplasms associated with the occurrence of bladder calculi, but that no-observed-adverse-effect levels (NOAELs) were determined, there was a correlation between the degree of epithelial hyperplasia and the induction of urinary bladder neoplasms, and the presence of dye particles in subepithelial layers was associated with the tumors of mesenchymal origin. They noted that bladder neoplasms occurred in both male and female rats, regardless of differences in chemical composition of the urinary bladder calculi, and that progression of urinary bladder tumors was halted in rats when Disperse Blue 1 treatment was discontinued. The authors believed this indicated that Disperse Blue 1 was not acting through a direct genotoxic mechanism.

They acknowledged that there was limited and weak evidence of mutagenicity in in vitro studies. However, it was noted there were no strong positive results obtained even at the highest concentrations tested. Additionally, there was a lack of dose–response, there were questions regarding solubility, and there was a possibility that mutagenic contaminants were present.

The authors evaluated the human safety of Disperse Blue 1 using two types of risk assessments: a biologically based approach and a conventional quantitative approach. In the first approach, an uncertainty factor of 1,000 was applied to the NOAEL in the NTP bioassay, which indicated a safe exposure level of 45–56 μg/kg per day. This value is 20 times greater than the estimated maximum lifetime average daily applied dose associated with use of semipermanent hair dyes (2.7

μg/kg per day). In the second approach, the linearized multistage model was applied to data on the incidence of leiomyomas and leiomyosarcomas. An exposure level corresponding to an upper limit of lifetime risk of 10^{-6} or 10^{-5} was 0.39 or 3.9 μg/kg per day, respectively. The latter value is 1.5 times greater than the estimated maximum lifetime exposure from semipermanent hair dye use. The authors concluded that "Because oral absorption is substantially more than dermal absorption, the actual margin of safety is most likely much greater than either of these comparisons suggest".

A similar conclusion was also reached in a separate evaluation by Weisburger (1989). Using the Decision Point Approach, it was noted that in the NTP (1986) bioassay Disperse Blue 1 could not be a carcinogen of the same types as 2-acetylaminofluorene or 4-aminobiphenyl or aflatoxin B1. The effects on the kidneys and urinary bladder of rats were not observed in the study with mice. Mutagenicity test results indicated a weak mutagenic response, but the author stated that contaminants (most notably nitrotriaminoanthraquinone) may be responsible since the chemical structure of Disperse Blue 1 is not typical of a genotoxic carcinogen. It was concluded that "Disperse Blue 1 cannot be considered to have intrinsic carcinogenic activity or potency". It was further stated that "This conclusion supports the additional view that under conditions of actual use, this chemical does not present a carcinogenic risk to humans".

Carcinogenicity of Structurally Related Compounds

The NTP's report on the carcinogenicity of Disperse Blue 1 identified three structurally related compounds that were also carcinogenic to animals: 2-aminoanthraquinone, 1-amino-2-methylanthraquinone, and 2-methyl-1-nitroanthraquinone (NTP, 1986). In a bioassay conducted by the National Cancer Institute (NTIS, 1978*a;* Murthy et al., 1979), 2-aminoanthraquinone was carcinogenic following dietary administration to male Fischer 344 rats, causing hepatocellular carcinomas and neoplastic nodules of the liver. It also caused hepatocellular carcinomas in male and female $B6C3F_1$ mice, and was associated with malignant lymphomas in female mice.

NCI also conducted bioassays for carcinogenicity with the other two compounds. 1-Amino-2-methylanthraquinone was carcinogenic to male and female Fischer 344 rats, causing hepatocellular carcinomas in both sexes, and renal neoplasms in male rats. This compound also increased the incidence of hepatic neoplasms in female $B6C3F_1$ mice (NTIS, 1978*b;* Murthy et al., 1979). In the bioassay of 2-methyl-1-nitroanthraquinone, this compound induced hepatocellular carcinomas in male Fischer 344 rats. An increased incidence of subcutaneous fibromas and of neoplasms of the nonglandular stomach and urinary bladder of both male and female rats were also associated with the administration of 2-methyl-1-nitroanthraquinone (NTIS, 1977; Murthy et al., 1979).

CLINICAL STUDIES

Dermal Irritation

One of 15 patients with contact dermatitis from textile dyes in trousers had a positive patch-test reaction to Disperse Blue 1 (Sim-Davies, 1972).

EPIDEMIOLOGY

Between 35 and 45% of American women dye their hair, often at monthly intervals, over a period of years (McEwen, 1993). This estimate is drawn from market research data on hair dye product use, generally from females aged 15 to 60.

A number of epidemiologic studies have investigated the association between cancer and occupation as a hairdresser or barber, or between cancer and personal use of hair dyes. The World Health Organization's International Agency for Research on Cancer (IARC) empaneled a Working Group on the Evaluation of Carcinogenic Risks to Humans to review all available data on these issues. The Working Group met October 6–13, 1992, in Lyon, France (IARC, 1993).

The charge to the IARC Working Group was to ascertain that all appropriate data had been collected and were being reviewed; to evaluate the results of the epidemiologic and experimental studies and prepare accurate summaries of the data; and to make an overall evaluation of the carcinogenicity of the exposure to humans.

The IARC Working Group concluded that "There is *inadequate evidence* that personal use of hair colourants entails exposures that are carcinogenic." Hence, "Personal use of hair colourants *cannot be evaluated as to its carcinogenicity (Group 3).*" The IARC Working Group also concluded that "There is *limited evidence* that occupation as a hairdresser or barber entails exposures that are carcinogenic." Hence, "Occupation as a hairdresser or barber entails exposures that *are probably carcinogenic (Group 2A)*" (IARC, 1993). The Expert Panel concludes that the relevance of the occupational data and conclusion for individuals using hair dyes is unclear.

SUMMARY

Disperse Blue 1 is an anthraquinone color using in hair dyes, colors, and rinses. In vitro dermal penetration studies using the skin from miniature pigs indicated that this compound is poorly absorbed. The oral LD_{50} for Disperse Blue 1 was >3,000 mg/kg for rats and >2,000 mg/kg for mice. In short-term and subchronic oral toxicity studies, rats and mice fed Disperse Blue 1 had reduced final body weights, were inactive, excreted blue urine, and had blue organs at necropsy. Typical histopathologic findings included accumulation of the dye in the renal tubules, hyperplasia of the renal pelvis epithelium, nephropathy, chronic inflammation and/or hyperplasia of the transitional epithelium of the urinary bladder, and calculi of the urinary tract.

In a chronic study, no adverse effects were observed when dogs were fed a formulation containing 1.54% Disperse Blue 1.

The irritation threshold for Disperse Blue 1 was >10%, and Disperse Blue 1 was a moderate sensitizer in studies with guinea pigs.

No adverse reproductive or teratogenic effects were observed in feed or gavage studies of dye formulations containing Disperse Blue 1.

In Ames tests, Disperse Blue 1 was mutagenic to some strains of *Salmonella typhimurium*, but conflicting results were sometimes obtained between studies.

Positive results were also obtained in a L5178Y mouse lymphoma cell mutation assay, in a chromosome aberration test, and in a sister chromatid exchange test.

In a carcinogenicity bioassay, Disperse Blue 1 was carcinogenic for male and female rats. It increased the incidence of transitional cell papillomas and carcinomas, of leiomyomas and leiomyosarcomas, and of squamous cell papillomas and carcinomas of the urinary bladder. Equivocal results were obtained in test with male mice, and negative results were found with female mice.

In another study, male and female rats developed lesions of the urinary bladder and kidneys, which were associated with dye deposits and calculi. However, no sarcomas of the bladder wall or epithelial neoplasms were observed.

A dermal carcinogenicity study testing two nonoxidative hair dye formulations containing Disperse Blue 1 was negative.

Risk assessments using both biologic and quantitative approaches indicated that Disperse Blue 1 does not pose a carcinogenic risk to humans because safe exposure concentrations determined in the NTP bioassay were significantly greater than the estimated maximum lifetime average daily applied dose associated with the use of semipermanent hair dyes.

DISCUSSION

The CIR Expert Panel noted both the mutagenicity of Disperse Blue 1 and the urinary bladder neoplasms observed in chronic feeding studies with Fischer 344 rats. They noted that these neoplasms appeared to be due to the formation of bladder calculi rather than a genotoxic mechanism. Such bladder calculi, while commonly seen in rats, do not appear to form in humans. Both biologic and quantitative risk assessments demonstrated that the safe exposure concentrations reported in the NTP bioassay were significantly greater than the estimated maximum lifetime exposure associated with the use of semipermanent hair dyes.

The Expert Panel also noted these neoplasms were not found in dermal carcinogenicity studies in mice. Disperse Blue 1 also appears to be poorly absorbed in in vitro studies. Based on these data and due to the fact that exposure to hair dyes is brief, the Expert Panel concluded that Disperse Blue 1 is safe for use in hair dyes at concentrations up to 1%.

CONCLUSION

Based on the animal and use data presented in this report, the CIR Expert Panel concludes that Disperse Blue 1 is safe for use in hair dyes at concentrations up to 1%.

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