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Final Report on the Safety Assessment of HC Blue No. 2[1]

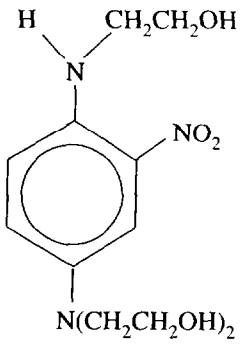
Abstract: The aromatic amine HC Blue No. 2 is used as a colorant exclusively in hair dyes. Current information indicates this ingredient is usually found in hair dyes at a concentration of ~1.7%. Studies in volunteers in which HC Blue No. 2 at use concentrations was applied to the scalp, <0.1% was absorbed over a period of 30 days. National Toxicology Program oral feeding bioassays in rats and mice shows the ingredient to be relatively nontoxic. Animal studies indicate no evidence of dermal irritation, sensitization, or photosensitization, and no ocular irritation. Whereas HC Blue No. 2 is mutagenic, it was not carcinogenic in rats or in two mouse strains. Clinical data indicate minimal irritation and no sensitization. On the basis of the available data, it is concluded that HC Blue No. 2 is safe as used in cosmetic formulations (hair dyes). **Key Words:** HC Blue No. 2—Safety—Cosmetic use—Hair dye—Rat—Mouse—Human—Chemistry—Oral toxicity—Mutagenicity—Carcinogenicity.

HC Blue No. 2 is a hair colorant used exclusively in hair dyes. The following is a summary of data available to the Cosmetic Ingredient Review (CIR) concerning the chemistry, cosmetic use, oral toxicity, mutagenicity, and carcinogenicity of this compound.

CHEMISTRY

Definition and Structure

HC Blue No. 2 (CAS No. 33229-34-4) is an aromatic amine that conforms to the following formula:



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

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Other names for HC Blue No. 2 include ethanol, 2,2'-[[4-[(2-hydroxyethyl)amino]-3-nitrophenyl]imino]Bis-; 2,2'-[[4-[(2-hydroxyethyl)amino]-3-nitrophenyl]imino]bisethanol; and N1,N4,N4-tris(2-hydroxyethyl)-2-nitro-*p*-phenylenediamine (Estrin et al., 1982).

Chemical and Physical Properties

Two lots of HC Blue No. 2 were analyzed by the National Toxicology Program (NTP, 1985). The compound has a molecular weight of 285 and appears as a dark blue microcrystalline or amorphous powder with a melting range of 83.5–90°C in one lot and 93–98°C in another. Infrared and nuclear magnetic resonance spectra, as well as thin-layer chromatography and high-performance liquid chromatography (HPLC) analyses, are available. HC Blue No. 2 had a major absorption peak at 263 nm in the UV and a secondary peak at 531 nm in one lot, and 264 nm and 534 nm in another, respectively. A third spectra confirmed the secondary peak at 531 nm [Cosmetic, Toiletry, and Fragrance Association (CTFA), 1993a].

Method of Manufacture and Impurities

Two methods for the preparation of HC Blue No. 2 were made available (CTFA, 1993a). One method, a two-step process, involves reacting 4-fluoro-3-nitroaniline with ethylene oxide to produce 4-fluoro-3-nitro-*N,N*-bis(2-hydroxyethyl)aniline, which in turn is reacted with monoethanolamine. HC Blue No. 2 synthesized in this fashion is >98% pure, with the major impurity being water. The second method discussed was the hydroxyethylation of 2-nitro-*p*-phenylenediamine, which had as its major impurity N^4,N^4-bis(2-hydroxyethyl)-2-nitro-*p*-phenylenediamine.

Purity determination by chromatographic analysis on HC Blue No. 2 (grade not specified) found ten impurities in one batch and five impurities in another batch. The impurities were not characterized. The purity of one lot was ~98%. HC Blue No. 2 was stable when stored for 3 years (NTP, 1985).

Cosmetic Use

The only reported use of HC Blue No. 2 in cosmetics is in hair dyes. Data submitted to the Food and Drug Administration (FDA) in 1993 by cosmetic firms who participated in the voluntary cosmetic registration program indicated that HC Blue No. 2 was used in 136 hair dyes and colors (requiring a cautionary statement), 7 hair tints, and 2 other hair coloring preparations (Table 1) (FDA, 1993). One source reported a usual cosmetic use concentration of 1.7% (CTFA, 1993a).

Hair-coloring formulations containing HC Blue No. 2 are applied to or may come in contact with hair, skin (particularly the scalp), eyes, and nails. Individuals dyeing their hair may use such formulations as often as once a week. Hairdressers may come in contact with products containing HC Blue No. 2 several times a day. Semipermanent hair dyes are usually applied in a shampoo base and contain thickeners, alkalizers, and foam stabilizers. Permanent hair dyes contain

TABLE 1. *Product formulation data (Food and Drug Administration, 1993)*

Product category	Total no. in product category	Total no. containing ingredient
Hair dyes/colors (requiring a cautionary statement)	811	136
Hair rinses	15	7
Other hair coloring preparations	207	2
1992 Totals		145

couplers and an oxidant in addition to the primary intermediate (the actual dye). Users may be exposed to reactive intermediates as well as to unreacted dyes (Corbett and Menkart, 1973).

The oxidative or permanent hair dyes containing the HC Blue No. 2, as "coal tar" hair dye products, are exempt from the principal adulteration provision and from the color additive provision 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 (Federal Register, 1979). In order to be exempt, the following caution statement must be displayed on all coal tar hair dye products:

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

Patch test instructions call for a 24-h patch on the skin of the user with the intermediates and hydrogen peroxide mixed in the same manner as in use. This test is to be performed prior to each and every application of the hair dye (Corbett and Menkart, 1973).

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 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.
>
> Since the recommendation of 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 of 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; Eiermann et al., 1982; Adams et al., 1985). Basically, 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 at 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.

International Use

The Scientific Committee on Cosmetology (1991) ruled that HC Blue No. 2 could be used in semi-permanent hair dyes at concentrations up to 2.8%.

GENERAL BIOLOGY

Absorption

Wolfram and Maibach (1985) examined the percutaneous absorption and excretion of a hair dye formulation containing 1.77% HC Blue No. 2 enriched with ^{14}C–HC Blue No. 2 (378.6 μCi/mg). The radioactive label was in the ring structure of HC Blue No. 2. The dye lotion was applied to the hair and scalp of four volunteers. The dye was worked into the hair and a plastic turban wrapped around the head. The dye was left on the hair for 30 min, after which the plastic wrap was removed, the hair was rinsed and then blotted dry. Urine samples were taken on Days 1, 10, 20, and 30; stratum corneum tape strip samples were taken directly after application and at 6 h. The average cumulative dose absorption increased over 30 days following application to a concentration of 0.09% of the total radioactivity applied. The time required for 50% excretion of the radioactivity in urine was 52 h; for comparison purposes, the equivalent figure for HC Blue No. 1 was 138 h. The radioactivity in the stratum corneum at the first stripping was 0.01% of the applied radioactivity. The second stripping resulted in no decrease in radioactivity in the 6-h interval. These data are different from HC Blue No. 1, a close homolog to HC Blue No. 2, where the 6-h stripping had a loss of 16% of the radioactivity seen initially. The authors conclude that HC Blue No. 2 is bound strongly to the stratum corneum. The authors postulate that loss of the stratum corneum through natural desquamation plays an important role in the bioavailability of HC Blue No. 2.

Metabolism

In Vivo Metabolism

A single oral gavage dose of 20 μl/g body weight radioactive HC Blue No. 2, ^{14}C uniformly distributed within the ring structure, was given to four female $B6C3F_1$ mice in a comparative study of the metabolism of HC Blue No. 2 and HC Blue No. 1. For the next 18 h, urine was collected over thymol crystals and feed was withheld from animals. The urine was filtered (2.2-μm pore size) and stored frozen (−70°C) prior to being counted for radioactivity and analyzed by HPLC. An average of 43 ± 10% of the initial radioactivity was recovered in the urine. Ac-

cording to the HPLC analysis, <5% of the initial HC Blue No. 2 radioactivity was excreted as parent compound, the remainder as more polar metabolites, the majority in one peak (Kari et al., 1990). That metabolite was determined to be 4-{N^4,N^4-bis(2-hydroxyethyl)amino}-2-nitrophenylglycine (CTFA, 1993a). This contrasts with the results for HC Blue No. 1 in which <5% of the initial radioactivity appeared as the parent compound, but the metabolites appeared in three major peaks totaling >70% of the recovered label. The authors concluded that there is a clear difference in the metabolism of HC Blue No. 1 and HC Blue No. 2, possibly explaining the difference in carcinogenic activity (Kari et al., 1990).

In Vitro Metabolism

The same radioactive HC Blue No. 2 as used in the previous experiment was added to isolated mouse hepatocytes in another comparison of HC Blue No. 2 and HC Blue No. 1 metabolism (Kari et al., 1990). HC Blue No. 2 in dimethyl sulfoxide (DMSO) was added to 2 ml of hepatocyte suspension to a concentration of 200 μM. DMSO was 1% of the final volume. Viability decreased ~55% independent of whether HC Blue No. 2, HC Blue No. 1, or DMSO control was added. Metabolic processes were stopped at each sample point by repeatedly (three times) freezing and then thawing the sample (samples stored at −70°C prior to HPLC analysis). The active hepatocytes metabolized ~25% of the HC Blue No. 2; heat-inactivated cells did not produce water-soluble metabolites. One major metabolite peak, resistant to β-glucuronidase and more polar than the parent compound, was found. A major difference with the metabolism of HC Blue No. 1 was that one of the three peaks found for HC Blue No. 1 was sensitive to β-glucuronidase, suggesting that peak was a glucuronide conjugate.

TOXICITY

Oral Toxicity

Acute Toxicity

Doses of 31, 62, 125, 250, and 500 mg/kg of HC Blue No. 2 were mixed with 1% carboxymethylcellulose ether sodium salt in saline and administered once, by gavage, to F344/N rats, five animals of each sex per dose group. The same method was used for $B6C3F_1$ mice at doses of 62, 125, 250, 500, and 1,000 mg/kg. The animals were weighed on the date of administration and observed twice daily for 15 days, and then weighed again. All of the animals survived to the end of the study. Mean body weight changes were not affected by the test material. A blue discoloration of the urine was observed in all dosed rats on Day 1 but not afterwards. In mice, the bluish discoloration of the urine continued in some animals through day 4. Mice in the highest dose group had reduced activity for the first 4 days of the study (NTP, 1985).

In two studies, HC Blue No. 2 was administered by gavage in a 10% suspension in 3% acacia to groups of five rats. Doses were 1,250 and 5,000 mg/kg for male rats and 1,250, 2,500, and 5,000 mg/kg for female rats (one test had an additional 3,500

mg/kg female dose group). The oral LD_{50} of HC Blue No. 2 was between 1,250 and 5,000 mg/kg (CTFA, 1992).

Short-Term Toxicity

NTP (1985) performed 14-day feeding studies using F344/N rats and $B6C3F_1$ mice. Animals were given feed containing 0, 3,100, 6,200, 12,500, 25,000, and 50,000 ppm HC Blue No. 2 and water ad libitum for 14 days. Feed consumption was not measured. Animals were observed twice daily and weighed on Days 1 and 15. All of the animals survived to the end of the study. Decreases in body weight gains were seen in the rat and mouse of the 25,000- and 50,000-ppm dose groups. Violet urine was seen in all animals throughout the study. Various tissues of the dosed rats were discolored.

NTP (1985) performed another set of 14-day feeding studies exactly as before, except with a different batch of HC Blue No. 2. All of the animals survived to the end of the study. Male rats of the 50,000-ppm dose group had a weight depression of 23% compared with controls. In mice, there was little appreciable difference in weight gain between dosed and control animals. All animals had violet-colored urine throughout the study.

Subchronic Toxicity

NTP (1985) performed 13-week feeding studies using $B6C3F_1$ mice and F344/N rats. Groups consisted of 10 animals of each sex. The animals received feed containing 0, 3,100, 6,200, 12,500, 25,000, and 50,000 ppm HC Blue No. 2 and water ad libitum. All animals were checked twice daily; the moribund animals were killed, weights were measured, and clinical examinations were performed weekly. At the end of the study, necropsy and histopathological evaluation of a variety of tissues were performed. All animals survived to the end of the experiment. Body weight depressions were seen in male rats fed diets containing 6,200 ppm (12% of control), 12,500 ppm (14% of control), 25,000 ppm (15% of control), and 50,000 ppm (21% of control). HC Blue No. 2 did not affect the weight of female rats. There was a dose-related incidence of thyroid pigmentation in both male and female rats. Body weight depressions were seen in male (11.5% of control) and female (9.9% of control) mice fed a diet containing 50,000 ppm HC Blue No. 2. All animals had violet-colored urine and feces.

Chronic Toxicity

Two-year feeding studies on HC Blue No. 2 were performed using rats and mice by NTP (1985). For male F344/N rats and male $B6C3F_1$ mice, groups of 50 animals were given diets containing 0, 5,000, and 10,000 ppm HC Blue No. 2 for 103 weeks. Groups of 50 female F344/N rats and female $B6C3F_1$ mice were given diets of 0, 10,000, and 20,000 ppm HC Blue No. 2. Animals were observed twice a day and weighed and palpated weekly. At the end of the study, necropsy and histopathological evaluation on a variety of tissues were performed.

Dosed rats of both sexes had little to no difference in survival as compared with controls. Mean body weights, when compared with controls, were reduced in a

dose-dependent manner: high-dose male, 8%; low-dose male, 7%; high-dose female, 12%; low-dose female, 13%. Rats had a dose-related increase in hyperosteosis of the skull, and there was an increased incidence of histiocytosis of the lungs in dosed rats (NTP, 1985).

In the female mice high-dose group, survival was significantly decreased as compared with control groups. Survival of all other dose groups was not significantly different from controls. Mean body weights, when compared with controls, were reduced in dosed animals by the following amounts: high-dose male, 5%; low-dose male, 5%; high-dose female, 12%; low-dose female, 15%. There was a dose-related incidence in fibrous osteodystrophy in female mice. Hematopoiesis of the liver and spleen was increased in female high-dose mice (NTP, 1985).

Dermal Irritation

In two studies, a dose of 500 mg HC Blue No. 2 in an aqueous slurry was applied to the intact skin of five rabbits (ten rabbits total: three female, seven male). Sites were scored at 24 and 48 h for erythema and edema. The primary irritation index was 0 (out of 12) (CTFA, 1992).

Dermal Sensitization and Photosensitization

The photosensitization potential of HC Blue No. 2 was studied with use of Hartley albino guinea pigs. For 4 consecutive days during the first week of induction, 0.1 ml of 10% HC Blue No. 2 was applied to a 1.8-cm diameter area of eight female and eight male shaved and depilated guinea pigs (nuchal area). After 1 h, animals were irradiated with a 150-W Xenon Lamp with a WG-354 glass filter for 7 min, which was equal to one-half the minimal erythemal dose (MED) for UVA light in guinea pigs. During the second and third week of induction, HC Blue No. 2 was applied to the same sites as before. After 1 h, animals were irradiated with the same light source without the filter for 60–120 s, which is equal to 1 MED UVB light in guinea pigs. On Days 1 and 3 of these 2 weeks, animals were injected with 0.1 ml of Freund's complete adjuvant in saline in an area surrounding the test site. The challenge phase, 2 weeks after the completion of the induction phase, consisted of the application of 0.1 ml of 5% HC Blue No. 2 to three sites in the left lumbar area. One area was irradiated with ½ MED UVA light; another area, ½ MED UVB light; and the third area was not irradiated. Musk ambrette, 5%, was used as a positive control (four male and four female guinea pigs treated as described earlier). There was neither irritation, sensitization, nor photosensitization reaction to HC Blue No. 2; all animals had a photosensitization reaction to the musk ambrette (CTFA, 1992).

Ocular Irritation

In two studies, 100 mg of HC Blue No. 2 was instilled into the conjunctival sac of four rabbits (eight rabbits total). In two of the rabbits, the dosed eye was rinsed with 20 ml of distilled water 20 s after instillation. Eyes were examined 1 h after

instillation and 1, 2, 3, and 7 days after instillation. Minimal to moderate conjunctivae redness, lid swelling, and discharge were seen in all animals the first 24 h. Dosed eyes had returned to normal in all animals by 1 week postinstillation. HC Blue No. 2, by this method, was considered a questionable ocular irritant (CTFA, 1992).

In two studies, 0.1 ml of HC Blue No. 2 in a 10% suspension in 3% acacia was instilled into the conjunctival sac of four rabbits (eight rabbits total). In two of the rabbits, the dosed eye was rinsed with 20 ml of distilled water 20 s after instillation. Eyes were examined 1 h after instillation and 1, 2, 3, and 7 days after instillation. HC Blue No. 2 induced some redness and discharge within the first 24 h, but dosed eyes returned to normal by Day 2. HC Blue No. 2, by this method, was considered a non-irritant (CTFA, 1992).

DEVELOPMENTAL TOXICITY

A semi-permanent hair dye formulation containing 1.7% HC Blue No. 2 was cutaneously tested for teratogenic effects on pregnant Charles River CD rats. Twenty rats were shaved and 2 ml/kg of the formulation was applied to their backs on Days 1, 4, 7, 10, 13, 16, and 19 of gestation. A positive control group received acetylsalicylic acid by gavage, and three negative control groups were shaved but received no treatment. All animals were killed on Day 20. No significant differences in the mean number of corpora lutea, live fetuses, and resorptions per pregnancy were reported for the experimental animals. There were no significant changes in soft-tissue anomalies or skeletal variations between the fetuses of the treatment group and the negative controls (Burnett et al., 1976).

MUTAGENICITY

HC Blue No. 2 was mutagenic in *Salmonella typhimurium* strains TA97 and TA98 but not in strains TA100 or TA1535, both with and without the presence of Arcolor 1254-induced liver S9 metabolic activation (NTP, 1985).

HC Blue No. 2 was mutagenic in a mouse lymphoma L5178Y/TK$^{+/-}$ forward mutation assay in doses ranging from 75 to 600 μl/ml (NTP, 1985).

In another study, HC Blue No. 2 induced unscheduled DNA synthesis in primary hepatocytes of rat, mouse, hamster, rabbit, and monkey in a dose-related manner (Hill et al., 1990).

An in vivo micronucleus induction assay was performed with use of ICR and CD-1 mice of both sexes. Micronucleus induction activity was observed in the female ICR 1,000-mg/kg dose group at the 24 h harvest time. No activity was observed at the 48 h time point, nor in any other dose or sex group. In a second experiment, no mice in any dose group had an increase in micronucleus induction (Parton et al., 1990).

HC Blue No. 2 induced small but significant increases in sister-chromatid exchanges and chromosomal aberrations in cultured mouse hepatocytes (Kari et al., 1990).

Oberly et al. (1990) found that HC Blue No. 2 was potently mutagenic in *S. typhimurium* TA98, both with and without metabolic activation. HC Blue No. 2

was not mutagenic in strains TA100, TA1535, or TA1537 or in *Escherichia coli* WP2uvrA$^-$, in concentrations of 312.5–5,000 μg/plate. HC Blue No. 2 was mutagenic in a dose-dependent manner in the mouse lymphoma L5178Y TK$^{+/-}$ assay in the presence and absence of metabolic activation.

CARCINOGENICITY

The carcinogenicity of HC Blue No. 2 was studied by NTP (1985) in 2-year rat and mouse bioassays. (These are the same studies described in the Chronic Toxicity section of this report.) In male F344/N rats and in male B6C3F1 mice, groups of 50 animals were given diets containing 0, 5,000, and 10,000 ppm HC Blue No. 2 for 103 weeks. Groups of 50 female F344/N rats and female B6C3F$_1$ mice were given diets of 0, 10,000, and 20,000 ppm HC Blue No. 2. Animals were observed twice a day and weighed and palpated weekly. At the end of the study, necropsy and microscopic examination on a variety of tissues were performed.

In male rats, there was a dose-dependent increase in the incidence of C-cell carcinomas of the thyroid gland. When combined with the incidence of C-cell adenomas, however, this trend was not statistically significant (Table 2). Two high-dose female rats had mixed mesenchymal neoplasms of the kidney. There was a dose-dependent increase in all types of malignant lymphomas in male mice, including the lymphocytic type, but the incidence in the high-dose group was not significantly greater than the control group by pairwise comparisons (Table 3). Two high-dose male mice had squamous cell papillomas. NTP (1985) concluded that "there was no evidence of carcinogenicity in male and female F344/N rats or in male and female B6C3F$_1$ mice receiving HC Blue No. 2 in the diet."

Another chronic feeding study assessed the carcinogenicity of HC Blue No. 2 in Balb/c mice (CTFA, 1992). Mice, 48 per sex per group, were given 1% HC Blue No. 2 mixed into the diet administered ad libitum for 19 months and 23 months for male and female mice, respectively. A control group of 48 mice per sex was fed the basal diet. Animals were observed daily. Examinations for toxicological or pharmacological effects occurred weekly. Body weights were measured every week for the first 3 months and then every month thereafter.

Between Weeks 82 and 85, all mice were killed for necropsy. At Week 91, 10 female mice from the dosed group and 10 female mice from the control group were killed for necropsy. Between Weeks 97 and 99, the remainder of the female mice were killed for necropsy. Necropsy included an external examination, a gross

TABLE 2. *C-cell adenomas and carcinomas of the thyroid in male rats (National Toxicology Program, 1985)*

Neoplasm	Study group		
	Control	5,000 ppm	10,000 ppm
Adenoma	7/50	2/50	5/49
Carcinoma	0/50	3/50	5/49
Adenoma or carcinoma	7/50	5/50	10/49

TABLE 3. *Hematopoietic neoplasms in male mice (National Toxicology Program, 1985)*

Neoplasm	Study group		
	Control	5,000 ppm	10,000 ppm
Malignant lymphomas	1/50	5/48	8/49

examination of the organs in situ, and the removal for microscopic examination of any lesions, masses, or suspected tumors and all of the major organs.

In addition, blood was collected from five and 14 female mice in dose and control groups, respectively, for hematology and clinical chemistry assays. During the first year, 20 male mice from the control group and 8 male mice from the dosed group died or were killed moribund. This was attributed to a large degree to a urinary tract infection. During this time, one female from the control group and no female mice from the dosed group died. During the second year, 9 male and 12 female mice from the control group and 12 male and 8 female mice from the dosed group died or were killed moribund. Mean body weight gain was significantly reduced in both the male and female dosed group as compared with that in the control groups. Weight gains in the dosed groups, however, were never <90% of controls.

Animals that died between the end of the first year and the animals at terminal necropsy were examined for liver and lung masses. There were no significant differences between dosed and control groups (Table 4). At the week 91 termination, one female from the control group and two from the dosed group had lung masses. A darkening of the gall bladder was observed in nine of the 10 dosed females. At termination for the male and female mice, there were no significant differences in the number of masses in lungs and liver (Table 5).

The gall bladder in the majority of animals dosed with HC Blue No. 2 was blue or contained a blue fluid. The dosed group had a reduced mean weight of the heart. An increased incidence of nonsupportive periocholangitis of the liver was seen in dosed mice. No other significant differences were seen in the dosed group, including hematological and clinical chemistry parameters.

In another carcinogenicity study, semi-permanent hair dye formulation containing 1.7% HC Blue No. 2 was applied topically at a volume of 0.05 ml to the clipped intrascapular area of a group of 50 male and 50 female Swiss-Webster mice once weekly for 23 months. At 7 and 9 months, 10 male and 10 female mice from each group were killed and necropsied. Gross and microscopic examinations were made on all mice that died during or were killed at the termination of the exper-

TABLE 4. *Gross changes in liver and lungs in mice that died before termination (Cosmetic, Toiletry, and Fragrance Association, 1992)*

Group	No. of animals that died	Liver masses	Lung masses
Dosed male	12	1	4
Control male	9	0	1
Dosed female	18	2	5
Control female	21	2	5

TABLE 5. *Gross changes in liver and lungs in mice at termination (Cosmetic, Toiletry, and Fragrance Association, 1992)*

Group	No. of animals	Liver masses	Lung masses
Dosed male	28	0	3
Control male	20	2	8
Dosed female	30	4	3
Control female	24	1	3

iment. There were three control groups. The incidences of neoplasms in control and treated groups were similar. Carcinogenic effects were not induced by the hair dye formulation (Burnett et al., 1980).

CLINICAL ASSESSMENT OF SAFETY

A repeated insult patch test (RIPT) on various materials including HC Blue No. 2 was performed on 100 volunteers. During the induction phase, 0.1 ml of 3% HC Blue No. 2 gel was applied with a semi-occlusive patch (1.4-cm blotting paper) to intact skin every 48 h for a total of 10 applications. Sites were scored prior to each new application. Patches applied on a Friday were replaced and scored on the following Monday. An 11-day nontreatment period followed the induction period. The challenge phase consisted of a single 48-h patch on a previously untreated site. Sites were graded immediately and 24 h after removal of the patch. There was one questionable irritation reaction and no sensitization reactions to HC Blue No. 2. Another repeated insult patch test, performed exactly as the previous study, was done on a different group of 102 volunteers. There were four panelists with questionable irritation reactions but no sensitization reactions to HC Blue No. 2 (CTFA, 1992).

EPIDEMIOLOGY

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

A number of epidemiological 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 epidemiological 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 to individuals using hair dyes is unclear.

SUMMARY

HC Blue No. 2 is used in cosmetics in a total of 116 hair rinses and dyes.

In a radioactive tracer experiment in humans using commercial formulations with HC Blue No. 2, the absorption increased over 30 days to 0.09% of the total radioactivity applied. The time for 50% excretion of the radioactivity in the urine was 52 h; results of tape strip analysis was that 0.01% of the applied radioactivity was in the stratum corneum after application and 6 h later. In an oral dosing study (mice), ~40% of the radioactivity of a ^{14}C–HC Blue No. 2 dose was excreted in the urine. Of this 40%, only 5% was in the parent compound; the majority of the radioactivity was recovered in a fraction identified as 4-{N^4,N^4-bis(2-hydroxyethyl)amino}-2-nitrophenylglycine. Isolated hepatocytes transformed HC Blue No. 2 to the same single metabolite.

HC Blue No. 2 was relatively nontoxic in oral assays, including 2-year bioassays performed by NTP.

HC Blue No. 2 was neither a dermal irritant, sensitizer, photosensitizer, nor an ocular irritant in animal studies.

In both in vitro and in vivo assays, HC Blue No. 2 was a potent mutagen. The compound was not carcinogenic in F344/N rats, B6C3F$_1$ mice, or Balb/c mice.

In two RIPT studies, one out of 100 panelists and four of 102 panelists developed irritation reactions to HC Blue No. 2. No sensitization reactions were observed.

DISCUSSION

The CIR Expert Panel recognizes that HC Blue No. 2 is genotoxic in many systems. The Panel, however, is persuaded by the final assessments of NTP that there is no conclusive evidence that this ingredient is carcinogenic in rodents. The Expert Panel also noted the lack of irritation and sensitization potential in animals as well as the small number of reactions to HC Blue No. 2 in two RIPT studies.

CONCLUSION

On the basis of the available data presented in this report, the CIR Expert Panel concludes that HC Blue No. 2 is safe as used in cosmetic formulations.

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REFERENCES

Adams RM, Maibach HF, Glendenning WE, et al. (1985) A five-year study of cosmetic reactions. *J Am Acad Dermatol* 12:1062–9.

Burnett C, Goldenthal EI, Harris SB, et al. (1976) Teratology and percutaneous toxicity studies on hair dyes. *J Toxicol Environ Health* 1:1027–40.

Burnett C, Jacobs MM, Seppala A, Shubik P. (1980) Evaluation of the toxicity and carcinogenicity of hair dyes. *J Toxicol Environ Health* 6:247–57.

Corbett JF, Menkart J. (1973) Hair coloring. *Cutis* 12:190–7.

Cosmetic, Toiletry, and Fragrance Association (CTFA). (1992) Submission of unpublished data by CTFA (Received on 5/20/92). Acute oral (two studies), ocular irritation (four studies), primary dermal irritation (two studies), photosensitization (one study), RIPT (two studies), carcinogenicity (one study). (300+ pages)*

Cosmetic, Toiletry, and Fragrance Association (CTFA). (1993a) Submission of unpublished data by CTFA. (Received on 1/22/93) Concentration of use in cosmetics, method of manufacture, impurities, and UV spectra. (2 pages)*

Cosmetic, Toiletry, and Fragrance Association. (1993b) Personal correspondence from G. N. McEwen. (Dated: 12/6/93).

Eiermann, et al. (1982) Prospective study of cosmetic reactions. *J Am Acad Dermatol* 6:909–17.

Elder RL, ed. (1985). Final report on the safety assessment of *p*-phenylenediamine. *J Am Coll Toxicol* 4:203–66.

Estrin NF, Crosley PA, Haynes CR, eds. (1982) *CTFA Cosmetic ingredient dictionary*. 3rd ed. Washington, DC: Cosmetic Toiletry and Fragrance Association, 119.

Federal Register. (October 16, 1979) Cosmetic product warning statements; coal tar hair dyes containing 4-methoxy-*m*-phenylenediamine (2,4-diaminoanisole) of 4-methoxy-*m*-phenylenediamine sulfate (2,4-diaminoanisole sulfate). 44(201):59509–10.

Food and Drug Administration (FDA). (1993) Cosmetic product formulation data. FDA computer printout. Washington, DC: FDA.

Hill LE, Parton JW, Probst GS, Garriott ML. (1990) Mutagenicity evaluation of HC Blue No. 1 and HC Blue No. 2. II. Effect on the in vitro induction of unscheduled DNA synthesis in rat, mouse, rabbit, hamster, and monkey primary hepatocytes. *Mutat Res* 241:145–50.

International Agency for Research on Cancer (IARC). (1993) IARC monographs on the evaluation of carcinogenic risks to humans. Vol 57. Occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines. Lyon, France; IARC, 43–118.

Kari FW, Driscoll SM, Abu-Shakra A, et al. (1990) Comparative metabolism and genotoxicity of the structurally similar nitrophenylenediamine dyes, HC Blue 1 and HC Blue 2, in mouse hepatocytes. *Cell Biol Toxicol* 6:139–55.

National Toxicology Program (NTP). (1985) Toxicology and carcinogenesis studies of HC Blue No. 2. NTP TR 293. August 1985.

North American Contact Dermatitis Group. *Patch testing in allergic contact dermatitis*. American Academy of Dermatology, 1980.

Oberly TJ, Kokkino AJ, Bewsey BJ, Richardson KK. (1990) Mutagenicity evaluation of HC Blue No. 1 and HC Blue No. 2. III. Effects in the *Salmonella typhimurium/Escherichia coli* reversion assay and the mouse lymphoma L5178Y $TK^{+/-}$ forward mutation assay. *Mutat Res* 241:151–9.

Parton JW, Beyers JE, Garriott ML. (1990) Mutagenicity evaluation of HC Blue No. 1 and HC Blue No. 2. I. Effect on the induction of micronuclei in mouse bone marrow cells. *Mutat Res* 241: 139–44.

The Scientific Committee on Cosmetology. (1991) Report of the Scientific Committee on Cosmetology concerning some hair dyes: N1-N4-N4-Tris(2-hydroxyethyl)-1,4-diamino-2-nitrobenzene. Commission of the European Communities. March 1, 1991, 15–21.

Wolfram LJ, Maibach HI. (1985) Percutaneous penetration of hair dyes. *Arch Dermatol Res* 277: 235–41.

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