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Final Report on the Safety Assessment of H.C. Red No. 1[1]

Abstract: H.C. Red No. 1 is an aromatic compound used as a colorant in semipermanent hair dyes and colors. This ingredient is reportedly used in almost 50 products; one manufacturer reports current use concentrations of ≤0.5%. These products will generally have a warning statement and patch test instructions that should be followed to determine whether each individual user is sensitive to the product before use. In a study performed using human female cadaver skin, the percutaneous absorption of H.C. Red No. 1 was linear for the first 4 h, with total absorption of 1.68% after 48 h. The oral median lethal dose was between 2.5 and 5.0 g/kg for male rats and 0.625 and 1.25 g/kg for female rats. Short-term oral feeding of H.C. Red No. 1 to rats had effects on several organ weights and resulted in liver and splenic lesions. Dermal exposure to almost twice the oral concentration produced no evidence of toxicity. In rabbits, H.C. Red No. 1 was not a dermal irritant, but it was a mild ocular irritant. H.C. Red No. 1 was a contact sensitizer, but not a photosensitizer, in guinea pigs. No reproductive or developmental toxicity was observed when a formulation containing 0.15% H.C. Red No. 1 was applied dermally to rats, and neither fetotoxic nor teratogenic effects were seen in rats fed ≤0.1% H.C. Red No. 1. No evidence of mutagenic potential was seen in most bacterial and mammalian assays. No carcinogenic effects were reported for mice dosed dermally with H.C. Red No. 1, but several possible effects were seen in a rat skin-painting study with 0.15% H.C. Red No. 1, including liver enlargement, parathyroid and hepatocellular hyperplasia, hepatocellular hypertrophy, hyperkeratosis in several locations, and dermatitis. Whether these effects were compound-related was unclear. A repeated-insult patch test of a 3% slurry of H.C. Red No. 1 completed on 103 individuals with normal skin reported one possible and one definite sensitization reaction. Because the ingredient is used at a low concentration and very little is actually absorbed, the oral exposure data using concentrations of ≤0.1% represent a much higher exposure than would occur through the skin. The general absence of toxicity in such oral studies supports the safety of use of H.C. Red No. 1 in hair dye formulations at concentrations of ≤0.5%. **Key Words:** H.C. Red No. 1—Hair dye—Mild ocular irritation—Contact sensitization.

H.C. Red No. 1 is a color additive that functions as a colorant in hair dyes and colors (Wenninger and McEwen, 1992).

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

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CHEMISTRY

Definition and Structure

H.C. Red No. 1 (CAS No. 2784-89-6) is the hair color that conforms to the formula shown in Fig. 1 (Wenninger and McEwen, 1993). H.C. Red No. 1 is also known as 4-amino-2-nitrodiphenylamine (Wenninger and McEwen, 1993; Clairol, 1994*a*); 2-nitro-*N'*-phenyl-1,4-benzenediamine (Wenninger and McEwen, 1993; Clairol, 1994*b*); 2-nitro-*N'*-phenyl-1,4-benzenediamine (Clairol, 1994*a*); 1,4-benzenediamine, 2-nitro-*N*(1)-phenyl-; *p*-phenylenediamine, 2-nitro-*N*(1)-phenyl-; and 2-nitro-4-aminodiphenylamine (Chemline, 1994).

Physical and Chemical Properties

H.C. Red No. 1 is a dark brown crystalline material, molecular weight 229, that is soluble in ethanol and insoluble in water, with a melting point in the range of 98–101°C (Clairol, 1994*b*). The empirical formula for H.C. Red No. 1 is $C_{12}H_{11}N_3O_2$.

Manufacture and Production

The following three-step process is used in the manufacture of H.C. Red No. 1: acetylation of 4-fluoro-3-nitroaniline, condensation with aniline, and hydrolysis (Clairol, 1994*a*). Published data on the ultraviolet absorbance of H.C. Red No. 1 were not found.

Analytical Methods

Published analytical methods data on H.C. Red No. 1 were not found.

Impurities

It is specified that H.C. Red No. 1 must exist as a minimum of 95% H.C. Red No. 1, with a maximum of 2% each of ash and 4-acetamino-2-nitrophenylamine (Clairol, 1994*a*).

USE

Cosmetic

H.C. Red No. 1 is reported to function as a hair colorant in hair dyes and colors (Wenninger and McEwen, 1992). Product formulation data submitted to the Food and Drug Administration (FDA) in 1994 reported that H.C. Red No. 1 is used in 47 cosmetic formulations (see Table 1) (FDA, 1994). H.C. Red No. 1 is reported

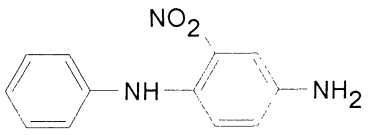

FIG. 1. Chemical formula for H.C. Red No. 1 (Wenninger and McEwen, 1993).

TABLE 1. *Product formulation data for H. C. Red No. 1*[a]

Product category	Total no. formulations in category	Total no. formulations containing ingredient
Hair dyes and colors	1,458	47

[a] From the FDA (1994).

to be used in semipermanent hair colors at a concentration of ≤0.5% (Clairol, 1994*b*; 1994*c*). Hair coloring formulations are applied to or may come in contact with hair, skin (particularly at the scalp), eyes, and nails. Individuals who dye 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 H.C. Red No. 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 Cosmetic Ingredient Review 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.
>
> 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; Eiermann 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.

Published data on the international cosmetic use of H.C. Red No. 1 were not found, nor were there any noncosmetic uses.

GENERAL BIOLOGY

Percutaneous Absorption

The rate of percutaneous absorption of ^{14}C-H.C. Red No. 1 from a commercial semipermanent dye base was examined using human female cadaver skin mounted in static Franz diffusion chambers (Clairol 1994*d*). ^{14}C-H.C. Red No. 1 was mixed with H.C. Red No. 1 to achieve a concentration of 1%, and the specific activity was equivalent to 0.156 μCi/mg. Skin integrity was assessed by determining the permeation rates of $^{3}H_2O$ over a 1-h period; the acceptable range was $\leq$1.5 mg/cm^2/h. The dye was applied to the stratum corneum side of the skin at a dose of 10 mg/cm^2 for 30 min, after which the dye was removed by rinsing with 2 ml of water. After 1, 2, 4, 6, 8, 24, 30, and 48 h, 200-μl samples were withdrawn from the receptor chamber and assayed for carbon 14 by scintillation counting. The rate of percutaneous absorption was linear for ~4 h following exposure; the rate of absorption was then reduced and essentially reached zero by 24 h. The total skin absorption at 24 and 48 h was 1.6 ± 0.01 and 1.68 ± 0.01%, respectively. (If data from one cell that had a much higher level of absorption was treated as an outlier, the mean total absorption was 1.36%.) The average skin permeability coefficient was 1.11×10^{-6} cm/h. Mass balance indicated that total radioactivity recovery was ~100%, with >95% being present in the rinsates used to remove the dye from the skin. The remainder of the radioactivity was distributed among the receptor fluid, the filter paper support, and the skin.

ANIMAL TOXICOLOGY

Acute Toxicity

Groups of five male and five female Sprague-Dawley rats were dosed orally with H.C. Red No. 1 as a 10% suspension in 3% acacia; the males were dosed with 1,250, 2,500, or 5,000 mg/kg and the females with 625, 1,250, and 5,000 mg/kg. The animals were observed for 7 days (Clairol, 1987*a*). Signs of toxicity were not recorded. All male and female rats of the 5,000 mg/kg dose group died within 24 h of dosing. All male rats in the other dose groups survived. Three female rats dosed with 1,250 mg/kg H.C. Red No. 1 died—one within 24 h, one within 2 days, and one within 4 days. No female rats of the 625 mg/kg dose group died within 7 days of dosing. The oral median lethal dose for male rats was between 2,500 and 5,000 mg/kg and for female rats was between 625 and 1,250 mg/kg H.C. Red No. 1.

Short-term Toxicity

Groups of Sprague-Dawley rats were fed H.C. Red No. 1 in the diet for 4 weeks in a pilot study in order to determine the dosages to be used in a longer-term

study, which will be described later in this review (Bristol-Myers Products, 1992). Twenty rats per group (10 per sex) were fed 0.05, 0.1, 0.2, 0.4, 0.6, or 0.8% w/w H.C. Red No. 1 in feed; a control group was fed untreated feed. Body weights and feed consumption were recorded weekly. The physical condition of the animals was observed daily, and pharmacologic and/or toxicologic observations were made weekly. After 4 weeks of dosing, necropsy was performed on the fasted animals. The liver, kidneys, and spleen from rats in each test group were weighed, and selected clinical chemistry evaluations were performed.

All animals survived until study termination. During the study, discolored urine and hair were observed in the test animals. When compared with control values, body weights and feed consumption were statistically significantly lower among animals of the 0.2–0.8% dose groups from weeks 1–4, with the exception of body weights and feed consumption for males of the 0.2% dose group during weeks 1 and 2 and feed consumption for all females during week 2. The only other statistically significant difference between test and control animals was a decrease in feed consumption by females of the 0.1% test group during week 1.

Statistically significant differences in many of the hematologic and clinical chemistry parameters were observed between test and control animals, especially those of the $\geq$0.1% test groups. Statistically significant increases were observed in the absolute and relative liver weights of males and females of the 0.4–0.8% dose groups, in the relative and absolute spleen weights of females of the 0.2–0.8% dose groups, in the relative kidney weights of females of the 0.4–0.8% groups, and in absolute kidney weights of males of the 0.6–0.8% dose groups. Compound-induced lesions were observed in the livers of female rats of the 0.8% test group and in the spleens of females of the 0.2 and 0.8% dose groups. Similar, but less prominent lesions were noted in the spleens of a few males does with 0.1% H.C. Red No. 1.

Subchronic Toxicity

Dermal

Groups of 12 New Zealand White rabbits, six males and six females per group, were used to determine the percutaneous toxicity of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 (Burnett et al., 1976). One milliliter per kilogram of mixture was applied undiluted twice weekly for 13 weeks to clipped sites on the dorsolateral aspects of the thoracic-lumbar area (one on each side of the midline), with the sites being alternated to minimize dermal irritation. The application sites on three animals per sex per group were abraded for the first dose of each week. The animals were restrained for 1 h following dosing, and the test site was then washed and rinsed. Three groups of negative control animals were treated in the same manner as the test animals, with the exception that no dye was applied. All animals were weighed weekly. Hematological, clinical chemistry, and urinary determinations were made at study initiation and after 3, 7, and 13 weeks. All animals were killed after 13 weeks and examined grossly. Various ratios of organ to body weight were determined, and a number of tissues were

examined microscopically. No evidence of compound-induced toxicity was found, no gross abnormalities were seen at necropsy, and no test article–related microscopic lesions were reported. No discoloration of the urine due to administration of the hair dye formulation was observed.

Oral

Male and female Sprague-Dawley rats were fed H.C. Red No. 1 at dietary concentrations of 0.01, 0.03, and 0.1% for 13 weeks; the 0.01% dose group consisted of 40 males and 45 females, and the 0.03 and 0.1% dose groups consisted of 40 males and 55 females (Bristol-Myers Products, 1992). A control group of 40 males and 45 females was fed normal feed. (Some of the animals used in this study were also used in teratology, dominant lethal, or micronucleus studies, which are summarized later in this report.) Ten rats per sex per group were dosed until week 27.

Animals were observed daily for death or moribundity and weekly for signs of toxicity. Body weights and feed consumption were measured weekly. At 13 weeks, blood was obtained by retroorbital bleeding from five males and five females of the control and high-dose groups to determine methemoglobin formation. At both the 13- and 27-week necropsy, blood was taken from 10 rats/sex/group for hematologic and clinical chemistry evaluation. The recovery animals were necropsied at week 28; the recovery animals consisted of the 20 males per group used in the dominant lethal study and 10 females of both the mid- and high-dose groups that were fed control feed between weeks 20 and 28.

No statistically significant differences in mean body weight among males were observed during weeks 0–13 of the study, and a statistically significant difference in mean body weight among females was observed only during week 10 between animals of the mid-dose and control groups. After the interim necropsy, body weights for males and females were similar throughout the remainder of the study. Feed consumption was statistically significantly increased during week 10 for males of the high-dose group. Feed consumption was statistically significantly lower for females of the low-dose group during week 1; for females of the mid-dose group during weeks 0–5, 7, and 10; and for females of the high-dose group during weeks 0–4 and 9. Females of the mid- and high-dose group consumed less than control females throughout weeks 0–13. Feed consumption was basically similar between test and control males and females throughout the remainder of the study. No statistically significant differences were observed in body weights of the male or female recovery rats compared with controls.

The urine of animals of all dose groups was rust-colored throughout the study. Several animals of the high- and mid-dose groups had discolored (red) hair by weeks 8 and 16, respectively. One female of the mid-dose group experienced rapid, labored breathing and had significant weight loss during week 9 and was moribund in week 10; multiple dark areas on the lungs, pale and mottled kidneys, a dark liver, and very little body fat were observed at necropsy. A male of the high-dose group (which was in the dominant lethal study) was found dead during week 21; red lungs, blood around the nose and mouth, a small spleen, and dark

material in the stomach were found at necropsy. No other statistically significant, compound-induced alterations were noted.

The only hematologic parameter that was statistically significantly different was an increase in the percentage of segmented neutrophils in females of the high-dose group after 13 weeks. Statistically significant differences in clinical chemistry values included an increase in total bilirubin in high-dose females after 13 weeks and increases in serum triglycerides and blood urea nitrogen in high-dose males and in serum sodium concentrations in mid- and high-dose females after 27 weeks. H.C. Red No. 1 did not induce methemoglobin formation.

There was a statistically significant increase in absolute and relative liver weights after 13 weeks and in relative kidney and relative liver weights after 27 weeks in males of the high-dose group. No statistically significant differences in organ weights were observed in recovery males or females. The only H.C. Red No. 1–related change noted at the 13-week necropsy was an increase in pigment in the spleens of females of the mid- and high-dose groups; the pigment was primarily composed of hemosiderin owing to the breakdown of red blood cells. The investigators described the increased deposition of pigment as an exacerbation of a normal occurrence in female rats. Only limited microscopic examination was made after 27 weeks because of a loss of tissues; no compound-induced alterations were recorded for the available tissues.

Dermal Irritation

The dermal irritation potential of H.C. Red No. 1 was assessed using six New Zealand White rabbits (Clairol, 1987*b*). H.C. Red No. 1, 500 mg, was applied as an aqueous slurry for a 1-sq-in area of shaved intact skin without occlusive patches. After 24 h, the residual material was removed, and the test site was scored according to the method of Draize at 24 and 72 h. Neither erythema nor edema was present 24 or 72 h after application; H.C. Red No. 1 was nonirritating upon nonocclusive application.

Dermal Sensitization

A guinea pig maximization test was performed according to the methods of Kligman-Magnusson using 10 female Hartley albino guinea pigs to determine the contact sensitization potential of H.C. Red No. 1 (Clairol, 1979*a*). A 4 × 6-cm area was clipped free of hair for induction, which consisted of an intradermal injection of 0.1% H.C. Red No. 1 in propylene glycol with Freund's complete adjuvant (FCA) followed 1 week later by the application of an occlusive patch of 25% H.C. Red No. 1 in propylene glycol for 48 h. The application area was pretreated with 10% sodium lauryl sulfate 24 h before patch application. The animals were first challenged 2 weeks later with a 24-h occlusive patch of 5% H.C. Red No. 1; 1 week later a second challenge was performed with a 24-h occlusive patch of 2% H.C. Red No. 1. Erythema and edema were observed at the test sites following challenge. There was evidence of contact sensitization to H.C. Red No. 1 in all animals at both the 5% and 2% challenges.

Because H.C. Red No. 1 produced a contact sensitization reaction in the pre-

vious test, the contact sensitization potential of H.C. Red No. 1 was assessed in an open epicutaneous test performed according to the method of Schultz using 10 female Hartley albino guinea pigs (Clairol, 1979*b*). Eighteen 0.5-ml applications of 3% H.C. Red No. 1 in aqueous vehicle containing 10% isopropanol, 2% Tween 80, and 2% hydroxyethylcellulose solution were made 5 days per week for 3 weeks and for 3 consecutive days during the fourth week to a shaved area on the left flank of each animal. The animals were challenged 2 weeks later with a 24-h application of the test material at a site on the opposite flank; observations were made after 24, 48, and 72 h. Contact sensitization was indicated by erythema, which was observed in seven animals after 24 h and in four animals after both 48 and 72 h; edema was not noted.

Photosensitization

The photosensitization potential of H.C. Red No. 1 was assessed using a group of 16 Hartley albino guinea pigs, eight per sex; a positive control group of eight guinea pigs, four per sex, was used (Clairol, 1987*c*). H.C. Red No. 1, 10%, was dissolved in 80% DAE (40% dimethylacetamide/30% acetone/30% ethanol) and 20% physiological saline; 5% musk ambrette was dissolved in the same vehicle and used as the positive control. The light source was a 150-W xenon lamp that emitted UVA, UVB, and visible light. The minimal erythemal doses (MED) for UVA and UVB were determined to be 14 min and 90 s, respectively. During week 1 of induction, 0.1 ml of the test material was applied to a 1.8-cm-diameter depilated site on the nuchal area for 4 consecutive days. One hour after application, the animals were irradiated with 0.5 MED of UVA; a WG-354 glass filter was used to remove UVB waves. Each animal was scored for dermal irritation according to the Draize scale 24 h after application. During weeks 2 and 3 of induction, 0.1 ml of the test material was again applied to the same depilated site for 4 days. One hour after application, the animals were irradiated with 1 MED of UVB. On days 1 and 3 of these weeks, each animal was given an intradermal injection of FCA in physiological saline (1:1) on four different sites surrounding the test area.

Two weeks after the last week of induction, the animals were challenged with three daily applications of a 5% H.C. Red No. 1 or musk ambrette solution on three different sites of the left lumbar area (1.8 cm diameter); each site was scored 24 h after each application for irritation. One hour after each application, the animals were irradiated with 0.5 MED of UVB at site 1, and with 0.5 MED of UVB at site 2; they received no UV irradiation at site 3. Two weeks after the initial challenge, the animals were rechallenged with 0.1 ml of a 1% H.C. Red No. 1 solution on a depilated 1-sq-in area on the right flank, which was not irradiated.

No erythema or edema was observed for any of the animals during induction; however, significant irritation was seen at the FCA injection site in all animals. The skin was discolored (rose colored) at the site of H.C. Red No. 1 application. Upon challenge, 13 of 16 animals responded (irritation score ≥ 1) at all three challenge sites, and there was no difference in the degree of irritation between these sites. On rechallenge, seven of 16 animals had irritation. H.C. Red No. 1 produced a contact allergic reaction, but not a photoallergic reaction, in guinea pigs.

Ocular Irritation

The ocular irritation potential of H.C. Red No. 1 was assessed using four New Zealand White rabbits in a modification of the Federal Hazardous Substances Act testing method using Draize scoring (Clairol, 1987*d*). One hundred milligrams H.C. Red No. 1 was placed in the conjunctival sacs of each rabbit. The eyes of two of the rabbits were rinsed 20 s following application. Mild effects were observed (scores of 1 and 2) and were limited to the conjunctiva (redness, swelling, and discharge). There was no corneal or iridial involvement. The unrinsed and rinsed eyes appeared normal by the second and third days, respectively, following application.

Reproductive and Developmental Toxicity

Dermal

Groups of 20 gravid Charles River CD rats were used to evaluate the teratogenic potential of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 (Burnett et al., 1976). The formulation was applied topically at a dose of 2 ml/kg to a shaved dorsoscapular area on days 1, 4, 7, 10, 13, 16, and 19 of gestation. Three negative control groups of rats were shaved but not dosed, and rats of a positive control group were dosed orally by gavage with 250 mg/kg acetylsalicylic acid on days 6–16 of gestation. Feed and water were available *ad libitum*. All animals were weighed on the days of dosing and killed on day 20 of gestation. The only reported observation was a change in color of the skin and hair at the site of application. No signs of toxicity were reported. Body weight gains and mean feed consumption were similar for animals of the treated and negative control groups. A semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 did not produce embryotoxic or teratogenic effects in Charles River CD rats.

A multigeneration reproduction study was conducted using groups of 80 Sprague-Dawley rats (40 rats per sex), which received topical applications of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 (International Research and Development Corporation, 1977). A dose of 0.5 ml was applied twice a week to a shaved area of the back ~1 in in diameter. (The initial dose was 0.2 ml per application, which was increased by increments of 0.1 ml per application weekly until reaching 0.5 ml per application.) Successive applications were made to adjacent areas on successive application days to minimize dermal irritation. Three negative control groups of rats were shaved but not dosed. When the rats were 100 days old, they were mated to produce an F_{1a} generation, which was eventually used in a carcinogenicity study. The F_0 generation was then reduced to 20 animals per group, remated to produce an F_{1b} generation, and then killed following weaning of the F_{1b} litters. Twenty male and 20 female rats per group were chosen from the F_{1b} litters and mated after 100 days to produce F_{2a} and F_{2b} litters. Five male and five female F_{1b} parents were necropsied after weaning of the F_{2b} litters.

Again following the same procedures, 20 male and 20 female F_2 parents per

group were selected from the F_{2b} litters and mated to produce F_{3a}, F_{3b}, and F_{3c} litters. After weaning the F_{3b} litters, one weanling per litter per group was necropsied; the pups of the F_{3a} and F_{3c} litters were killed after weaning. Parental generations were observed daily for changes in general behavior and appearance, and detailed observations were recorded weekly. Body weights and feed consumption were measured weekly. The pups were counted and weighed as a litter on days 0, 4, and 14 of lactation. On day 21 of lactation, the pups were counted, sexed, and examined for pharmacological effects.

Dermal reaction consisting of mild scabbing, fissuring, atonia, and a leathery texture occurred intermittently throughout the treatment period in each generation. No dose-related pharmacotoxicological signs were found, and body weight gains, feed consumption, and survival were comparable for treated and control rats in each generation. During week 61, sialoadenitis was noted in some test and control animals; this condition regressed at week 63 but was followed by an increased incidence of respiratory congestion in both test and control animals. The respiratory congestion persisted in the F_2 parents during the production of successive litters. Litter size and pup body weights were similar for test and control groups. Fertility, gestation, survival, and live birth indexes were comparable between test and control animals for the F_0, F_1, and F_2 parents. The F_2 parents had markedly reduced fertility indexes for the three separate matings, but there were no significant differences between the control and test groups with respect to fertility. The researchers did not report that respiratory congestion was a significant factor in the reduction of fertility indexes. The results of a special study established that the decreased fertility was due to reproductive tract changes in both the treated and control rats and therefore was not related to the test article. No gross or microscopic treatment-related lesions were observed in F_{1b} parental rats or F_{3b} weanling rats. The topical application of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 did not affect the reproductive performance of rats.

Oral

As reported earlier, animals from a subchronic feeding study were used in a developmental toxicity study (Bristol-Myers Products, 1992). After 15 weeks of dosing, 25 females per group (0, 0.01, 0.03, or 0.1% H.C. Red No. 1 in feed) were fed control diet throughout mating. Females were observed daily for general appearance and toxicity. Body weights were measured at study initiation and on days 6, 9, 12, 15, and 20, and feed consumption was determined for 10 gravid rats/group on days 11–12 and 19–20 of gestation. The animals were killed on day 20 of gestation. There were no statistically significant differences in body weights or feed consumption between the test and control groups during gestation; mean body weight gains were statistically significantly lower for dams of the high-dose groups during days 6–9 and days 0–9, 0–12, and 0–15. Gravid uterine weights and maternal carcass weights were not statistically significantly different between test and control groups. There were no statistically significant differences between test and control groups with regard to any cesarean-section observations or fetal

morphological examinations. H.C. Red No. 1 did not produce a fetotoxic or a teratogenic response.

MUTAGENICITY

An Ames test was performed using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, and TA98 in which H.C. Red No. 1 was assayed for mutagenic potential at concentrations of 25–5,000 μg in the presence of metabolic activation (Clairol, 1991). Positive and negative controls were used. H.C. Red No. 1 did not increase the number of revertants in any of the *S. typhimurium* strains in the presence of metabolic activation. An assay was performed to evaluate the potential of H.C. Red No. 1 to induce unscheduled DNA synthesis (UDS) in primary cultures of rat hepatocytes by measuring the incorporation of tritiated thymidine into DNA (Pharmakon Research International, Inc., 1993). H.C. Red No. 1 was tested in triplicate at a dose range of 0.1–2,500 μg/ml in dimethylsulfoxide (DMSO) with concurrent untreated, negative, and positive controls. Upon visual inspection, it was determined that 25 μg/ml was the highest dose to be scored owing to toxicity; cultures dosed with 1, 5, and 10 μg/ml were also evaluated. H.C. Red No. 1, 1–25 μg/ml, was negative in inducing UDS (repair) in rat primary hepatocytes.

A chromosomal aberration study was performed using Chinese hamster ovary (CHO) cells to evaluate the clastogenic potential of H.C. Red No. 1 (Integrated Laboratory Systems, 1993). H.C. Red No. 1 in DMSO was tested at a dose range of 7.5–100 μg/ml in the presence and absence of metabolic activation. Cyclophosphamide and mitomycin C were used as the positive controls in the presence and absence of metabolic activation, respectively. Without metabolic activation, chromosomal aberrations were evaluated only for the cultures treated with 7.5–25 μg/ml owing to excessive toxicity to the cultures dosed with 50–100 μg/ml. H.C. Red No. 1 did not induce a significant increase in the percentage of metaphase cells containing at least one aberration. The mitotic index (MI) was significantly decreased, but the percentage of polyploid metaphase cells was not significantly different. Based on pairwise comparisons, CHO cells dosed with 7.5–100 μg/ml in the absence of metabolic activation had a depressed MI. With metabolic activation, chromosomal aberrations were evaluated for the cultures treated with 50–100 μg/ml. Over this dose range, H.C. Red No. 1 induced a significant increase in the percentage of metaphase cells containing at least one aberration. Again, the MI was depressed, while the percentage of polyploid metaphase cells was not significantly altered. Using pairwise comparisons, the aberration response, consisting primarily of chromatid-type breaks and rearrangements, was significantly increased, and the MI was significantly depressed only for cultures dosed with 100 μg/ml in the presence of metabolic activation.

During the previously described subchronic feeding study, bone marrow smears were prepared from the femurs of five males per group (0, 0.01, 0.03, or 0.1% H.C. Red No. 1 in feed) at the time of the 13-week necropsy and processed for a micronucleus assay (Bristol-Myers Products, 1992). There were no statistically significant differences in the number of micronucleated polychromatic erythro-

cytes between the control and test groups. H.C. Red No. 1 did not have a clastogenic effect.

Animals from the previously described subchronic feeding study were also used in a dominant lethal study (Bristol-Myers Products, 1992). After 20 weeks of dosing, 20 males per group (0, 0.01, 0.03, or 0.1% H.C. Red No. 1 in feed), which were fed control feed throughout the remainder of the study, were mated with nondosed females. The males were weighed weekly and observed daily for general condition and signs of toxicity. Females were also observed daily; weighed on days 0, 12, and 17 of gestation; and killed on day 17 of gestation. No statistically significant differences in body weights of gravid females were observed between the test and control groups. There were also no statistically significant differences reported for any litter or reproduction parameters.

CARCINOGENICITY

A 23-month skin-painting study was performed using groups of 100 Eppley Swiss Webster mice (50 males and 50 females per group) to determine the carcinogenic potential of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 (Burnett et al., 1980). A 0.05-ml sample of the test solution was applied undiluted to a 1-cm^2/area of clipped skin of the interscapular region. A group of negative controls was shaved but not dosed. Observations were made daily, and body weights were measured monthly. After 9 months, 10 male and 10 female animals from each group were necropsied, and liver and kidney weights were determined. Gross and microscopic examinations were made for all animals found dead, killed due to moribund condition, or killed at study termination. Relative and absolute liver and kidney weights were not significantly different from control values. No dose-related neoplasms were observed. A semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 applied dermally for 23 months did not induce a carcinogenic effect.

F_{1a} generation Sprague-Dawley rats from a previously described reproduction study (International Research and Development Corporation, 1977) were used to determine the carcinogenic potential of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 (International Research and Development Corporation, 1979). Twice a week a dose of 0.5 ml of the hair dye formulation was applied topically to a shaved area of the back, (~1 in in diameter) of 120 rats, 60 per sex, for 12 months. (The initial dose was 0.2 ml per application, which was increased by 0.1 ml per application increments weekly until reaching 0.5 ml per application.) Successive applications were made to adjacent areas to minimize dermal irritation. Three negative control groups of 120 rats were shaved but not dosed.

The rats were observed daily for signs of toxicity and mortality; detailed observations were recorded weekly. Body weights were measured weekly for the first 14 weeks and monthly thereafter; feed consumption was determined weekly. Biochemical measures were determined from blood and urine samples that were collected from five male and five female fasted rats per group at 3, 12, 18, and 24 months. Five male and five female rats per group were killed after 12 months. No

signs of toxicity were observed. Test rats had a slightly greater incidence of skin lesions from various locations, including ulceration, scabbing, abscess formation, and thickening. Coloration of the hair and skin at the application site was observed in several treated rats, but it was not considered to be pathologically significant. Body weight gains, survival, hematological values, and biochemical measures were similar for rats of the treated and control groups. After 3, 12, and 24 months, the animals consistently had dark straw-colored urine; three and nine rats had dark brown urine at 12 and 18 months, respectively.

The incidence of enlarged and/or firm livers was slightly greater in the test group compared with the controls; this result was considered to be "possibly compound related." Other lesions considered to be possibly compound related for males and females of the test group include parathyroid gland hyperplasia, hepatocellular hypertrophy or hyperplasia, and hyperkeratosis and dermatitis from a variety of locations. There was a considerably higher incidence of this last effect. Several male test rats had hyperkeratosis and/or acanthosis involving the gastric mucosa, which was also possibly compound related.

The incidence of hematopoiesis in the livers of test rats was somewhat greater than that of all controls; the significance of this increase was not determined. For female test animals, the incidence of pituitary adenomas was significantly higher compared with females from two of the three control groups, and the incidences of mammary adenocarcinoma/mammary carcinoma were significantly higher compared with females in one of the three control groups; however, these differences were not considered biologically significant. Actuarial (life table) analyses did not report significant variations in indexes of tumor bearing in the test animals compared with the control groups by sex.

CLINICAL ASSESSMENT OF SAFETY

Sensitization

A repeated-insult patch test was performed to determine the sensitization potential of H.C. Red No. 1 (TKL Research, Inc., 1987). Of the 105 initial subjects, 103 completed the study. During the induction phase, H.C. Red No. 1 was applied as 0.2 ml of a 3.0% slurry in a bland base to the infrascapular area of the back under an occlusive patch for nine consecutive applications. Patches were removed 24 h after application, and the test sites were read either 48 or 72 h after application. The challenge patch was applied to a previously untested area 2 weeks after the last induction patch; the patch was removed after 24 h and scored 48 and 72 h after application. One subject had reactions indicative of presensitization, and another subject had reactions indicative of sensitization to H.C. Red No. 1.

Epidemiology

Between 35% and 45% of American women dye their hair, often at monthly intervals, over a period of years (Cosmetic, Toiletry, and Fragrance Association, 1993). This estimate is drawn from market research data on hair dye product use,

generally from females aged 15–60. A number of epidemiologic studies have investigated the association between cancer and occupation as a hairdresser or barber and 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 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 CIR Expert Panel concludes that the relevance of the occupational data and conclusion to individuals using hair dyes is unclear.

SUMMARY

H.C. Red No. 1, a color additive that functions as a colorant in hair dyes and colors, is a dark brown crystalline material. It is soluble in ethanol and insoluble in water. It is manufactured by a three-step process and must exist as a minimum of 95% H.C. Red No. 1. In 1994, data submitted to the FDA reported that H.C. Red No. 1 was used in 47 hair dye and color formulations; data received from industry reported that it is used at concentrations of $\leq$0.5% in oxidative and semipermanent hair dye formulations. Hair dyes containing H.C. Red No. 1, as coal tar hair dyes, 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 irritation.

The rate of percutaneous absorption of 1% H.C. Red No. 1 was linear for ~4 h following exposure. The total skin absorption at 24 and 48 h was 1.6 $\pm$ 0.01 and 1.68 $\pm$ 0.01%, respectively. The oral median lethal dose of H.C. Red No. 1 for male and female rats was between 2,500 and 5,000 mg/kg and between 625 and 1,2500 mg/kg, respectively. In a short-term oral study in which rats were fed $\leq$0.8% H.C. Red No. 1, some statistically significant differences in liver, spleen, and kidney weights were observed between some of the test and control groups. Dose-related lesions were observed in the livers and spleens of some female test rats; similar but less prominent splenic lesions were observed for some male rats. In a dermal subchronic toxicity study of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1, the urine of rats was not discolored, and no evidence of compound-induced toxicity was found. In an oral subchronic study in

which rats were fed ≤0.1% H.C. Red No. 1, animals of all dose groups had rust-colored urine throughout the study, the hair of some animals was discolored red, the liver and spleen weights of some males were increased, and there was an increase in splenic pigmentation in some dosed females after 13 weeks; H.C. Red No. 1 did not induce methemoglobin formation. An aqueous slurry of 500 mg H.C. Red No. 1 was nonirritating to rabbits when applied under nonocclusive conditions. H.C. Red No. 1 did produce contact sensitization in guinea pigs under both open and occlusive conditions, but it did not produce a photoallergic reaction. The application of 100 mg H.C. Red No. 1 in the conjunctival sacs of rabbit eyes resulted in mild effects.

The ability of a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 to induce developmental toxicity (including teratogenicity) or reproductive effects upon dermal application was examined. In a developmental toxicity study, the formulation caused a change in the application site skin and hair color, but it did not produce embryotoxic or teratogenic effects. The formulation did not affect reproductive performance in a multigeneration study. In an oral study in which rats were fed ≤0.1% H.C. Red No. 1, neither a teratogenic nor a fetotoxic response was observed. H.C. Red No. 1 produced no evidence of mutagenic potential in an Ames test, was negative in inducing UDS in rat primary hepatocytes, did not have a clastogenic effect in a micronucleus assay, and produced no dominant lethal effects. Based on pairwise comparisons, CHO cells dosed with 100 μg/ml and 7.5–100 μg/ml H.C. Red No. 1 had a depressed MI in the presence and absence of metabolic activation, respectively; in the presence of metabolic activation, the aberration response was also significantly increased.

A semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 did not produce carcinogenic effects in a 23-month mouse skin-painting study. However, in a second dermal carcinogenicity study with a semipermanent hair dye formulation containing 0.15% H.C. Red No. 1 using rats, discolored urine was reported, and the following, possibly compound-related alterations were observed: enlarged and/or firm livers, parathyroid gland hyperplasia, increased frequency of hepatocellular hypertrophy or hyperplasia, and increased incidence of hyperkeratosis and dermatitis from a variety of locations for male and female test rats, and hyperkeratosis and/or acanthosis involving the stomach mucosa in some male test rats. In a human repeated-insult patch test of a 3.0% slurry of H.C. Red No. 1, one subject had reactions indicative of presensitization, and another subject had reactions indicative of sensitization.

DISCUSSION

The CIR Expert Panel considered oral toxicity data in which animals were exposed to H.C. Red No. 1 in their diet at concentrations of ≤0.1%, as well as cutaneous absorption data showing that ~1.6% of applied H.C. Red No. 1 was absorbed through the skin. Based on this information and additional data summarized in the report, the Expert Panel was able to extrapolate the oral exposure to determine that H.C. Red No. 1 is safe as used at concentrations of ≤0.5%.

CONCLUSION

On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that H.C. Red No. 1 is safe as used in hair dye formulations at concentrations of ≤0.5%.

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