FINAL REPORT ON THE SAFETY ASSESSMENT OF HC ORANGE NO. 1[1]

HC Orange No. 1 is used as a colorant in semipermanent hair dyes. The highest concentration reported to be used is 0.15%, but information from manufacturers suggested that higher concentrations may be used in the future. Skin penetration through cadaver skin was 1.28% at 24 hours. In studies using rats, acute oral exposure studies produced little toxicity, and short-term toxicity studies produced reduced body weight and increased liver and kidney weights, relative to controls in animals fed 0.5% HC Orange No. 1. There was no evidence of reproductive or developmental toxicity in rats fed up to 1.25% HC Orange No. 1 or in a multigeneration study using rats in which 0.15% HC Orange No. 1 was painted on the skin. While evidence suggests this ingredient is a mild ocular irritant, no skin irritation, sensitization, or photosensitization was seen in animal or clinical tests. The preponderance of data (four out of five studies) indicate that this ingredient is not genotoxic. Hepatocellular and parathyroid hyperplasia were noted in the dermal carcinogenicity study, but the overall findings were clearly negative. Because the highest concentration tested that produced no significant sensitization in clinical tests was 3%, the Expert Panel concluded that safety could be assured only at levels ≤3%. The Expert Panel recognized that this concentration may be greater than that currently used in hair dye formulations.

HC Orange No. 1 is a hair colorant used in semipermanent hair dyes. The following report is a summary of the safety data on this ingredient.

CHEMISTRY

Definition and Structure

HC Orange No. 1 (CAS No. 54381-08-7) is the hair color that conforms to the formula shown in Figure 1. Other names for HC Orange No. 1 are 2-nitro-4′-hydroxydiphenylamine and 4-[(2-Nitrophenyl)Amino]Phenol (Wenninger and McEwen 1997).

Received 20 January 1998; accepted 30 April 1998.

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Susan N.J. Pang, former Scientific Analyst and Writer, and Monice Zondlo Fiume, Scientific Analyst/Report Management Coordinator, prepared this report.

Address correspondence to Monice Zondlo Fiume, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

1091-5818/98 $12.00 + .00

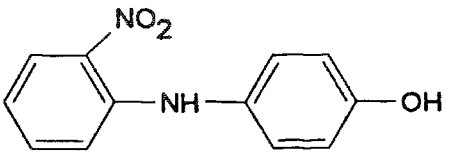

Figure 1. Chemical formula for HC Orange No. 1.

Physical Properties

HC Orange No. 1 is a dark orange fine crystalline material, insoluble in water, but soluble in ethanol (Clairol 1995).

Method of Manufacture

One reported method of manufacture of HC Orange No. 1 is the reaction of *o*-nitrochlorobenzene with *p*-aminophenol in isopropanol. The commercial product is reported to be >99% pure; the impurities are unknown (CTFA 1992). Another reported method is via the reaction of 2-fluoronitrobenzene with 4-aminophenol (Clairol 1995).

COSMETIC USE

HC Orange No. 1 is used as a color additive in hair dyes and colors (Wenninger and McEwen 1997). The product formulation data submitted to the Food and Drug Administration (FDA) in 1996 reported that HC Orange No. 1 was used in a total of 95 hair dyes and colors that require caution statements and patch test instructions (Table 1) (FDA 1996). Concentration of use values are no longer reported to the FDA by the cosmetic industry (FDA 1992). However, the ingredient is currently used by Clairol in semipermanent hair colors at concentrations up to 0.15% (Clairol 1995).

Hair coloring formulations containing HC Orange No. 1 are applied to or may come in contact with hair, skin (particularly at the scalp),

Table 1. Cosmetic product formulation data on HC Orange No. 1

Product category	Total no. formulations in category	Total no. of formulations containing ingredient
Hair dyes and colors	1612	95
1996 totals		**95**

Source. FDA, 1996.

eyes, and nails. 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.

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 skin irritation (FDA 1979). 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.
>
> 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.

GENERAL BIOLOGY

Dermal Absorption

The percutaneous absorption of a semipermanent hair dye containing ^{14}C-HC Orange No. 1 (specific activity: 38.1 μCi/mg) was determined in vitro. Human female cadaver split-thickness skin was mounted in static horizontal diffusion cells. ^{14}C-HC Orange No. 1 was mixed with nonradioactive HC Orange No. 1 and added to the hair dye base to obtain a dye concentration of 1.0% (0.130 μCi/mg). This mixture was applied (10 mg/cm^2) to the skin surface for 30 minutes, followed by rinsing. Samples of the receptor fluid were taken at 1, 2, 4, 6, 8, 24, 30, and 48 hours after the dye was removed. The amount of radioactivity found in these samples was used to quantify the percent of percutaneous absorption, rate of absorption, and the permeability coefficient.

The permeation rate, expressed as dose (%)/hour, peaked at 1.5 hours and rapidly decreased to a steady state level 6 hours later. The cumulative absorption at 24 hours was 1.28 ± 0.96% of the applied dose and the average flux was 1.36 μg/cm^2. At 48 hours, skin absorption was 1.41 ± 0.96% and average flux was 1.49 μg/cm^2. Mass balance determinations indicated that 98% of the applied radioactivity was recovered. Ninety-six percent of this amount recovered was in the rinsates and 0.42% remained in the skin (Clairol Research & Development Laboratories 1994).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Two groups of five male Sprague-Dawley rats were given a 10% suspension of HC Orange No. 1 in 3% aqueous acacia by gavage in doses of 1250 and 5000 mg/kg. A group of five female rats was given 5000 mg/kg. The animals were observed for 14 days. One male rat from the 5000 mg/kg treated group died on day 2. No other deaths occurred in any of the other groups. For both male and female rats, the median lethal dose and the minimum lethal dose were estimated to be >5000 mg/kg and >1250 mg/kg, respectively (CTFA 1987a).

Short-Term Oral Toxicity

HC Orange No. 1 was given in the diet to groups of 10 male and 10 female Sprague-Dawley rats at concentrations of 0.125, 0.5, 0.875, and

1.25% for 4 weeks. The animals were observed regularly for signs of toxicity and body weights were recorded. At the end of the study, all of the animals were killed and necropsied. Significantly lower body weights were observed in rats fed diets containing concentrations of 0.5% HC Orange No. 1 and greater. The relative liver weights were increased in male rats fed 0.875% HC Orange No. 1 and in both sexes fed 1.25%. Relative kidney weights were also increased in the female rats fed 1.25% HC Orange No. 1 (Loehr and Re 1990).

Subchronic Oral Toxicity

A combined subchronic toxicity and reproductive and developmental toxicity study was conducted by Bristol-Myers Products (1994). The latter results will be described later in this report. Groups of 30 male and 55 female Sprague-Dawley rats were fed 0.125, 0.4, and 1.25% HC Orange No. 1 in the diet for periods up to 6 months. Body weights and feed consumption were recorded throughout the study and observations for signs of toxicity were made regularly. Ten rats per sex from each group were killed after 3 months for hematology and clinical chemistry determinations, as well as histopathologic evaluation (3-mo subchronic study). After 14 weeks, 25 female rats from each group were switched to control diet and were mated to untreated males. Once insemination occurred, the females were returned to the test diet, which was given throughout the gestation period. All pregnant females were killed on day 20 of gestation and maternal and fetal effects were determined (reproductive and developmental toxicity study). The remaining rats were killed and necropsied after 6 months (6-month subchronic study).

Throughout the subchronic toxicity study, significant reductions in body weight were observed among the mid- and high-dose animals. Feed consumption was also reduced, achieving statistical significance at various points. The only consistent hematologic effects observed were reduced hemoglobin values and increased reticulocyte counts in female rats. The investigators speculated that these effects were related to pigment accumulation in the spleen. A dose-related (but not statistically significant) elevation in serum cholesterol was observed in female rats after both 3 and 6 months. A slight reduction in glucose levels and increases in blood urea nitrogen (BUN) and BUN/creatinine ratio were observed in the mid- and high-dose groups; these changes were likely related to the microscopic lesions observed in the kidneys and liver. At necropsy, changes in kidney weights and relative kidney weights were observed at 3 and/or 6 months for all treated male rats and the mid- and high-dose female rats. Dose-related microscopic changes were observed in the kidneys. In male rats, hyaline droplet formation was apparent

in all treatment groups after 3 months. There was an increase in the incidence and degree of thyroid follicular cell hypertrophy, associated with increased spleen weight, in all groups of treated male rats and in the mid- and high-dose groups of female rats. Varying degrees of follicular cell hypertrophy were also observed in the control rats. Pigment deposits were found in the spleens of treated rats and appeared to be related to increased erythrocyte turnover, as evidenced by the increased number of reticulocytes in high-dose males at 3 months and/or mid- and high-dose females at 3 and 6 months.

Dermal Irritation

An aqueous slurry of 500 mg HC Orange No. 1 was applied to the intact skin of six New Zealand white rabbits without occlusive patches. The slurry was removed after 24 hours. No evidence of erythema or edema was observed at 24 or 72 hours (CTFA 1987b).

Sensitization

In a sensitization study, a single intradermal injection of 0.1% HC Orange No. 1 in propylene glycol and Freund's complete adjuvant (FCA) was given to 10 female Hartley albino guinea pigs. One week after injection, an occlusive patch containing 25% HC Orange No. 1 in propylene glycol was applied to an area over the injection site which had been pretreated 24 hours earlier with 10% sodium lauryl sulfate. After a 2-week nontreatment period, 25% HC Orange No. 1 was applied to a previously untreated site. No evidence of contact sensitization was observed (Loehr 1979).

Photosensitization

The photosensitization potential of HC Orange No. 1 was tested using Hartley albino guinea pigs. Prior to the induction phase of the experiment, the minimal erythemal dose (MED) for UVA and UVB in guinea pigs was determined to be 14 minutes and 90 seconds, respectively. The light source used was a 150W Xenon Lamp (Solar Light Company, Philadelphia, PA), which emitted UVA (320–410 nm), UVB (280–320 nm), and visible light waves ($\geq$410 nm). The nuchal areas of all the test animals were shaved and depilated 24 h prior to induction, and the animals were shaved daily throughout the experiment. A positive control group of four male and four female guinea pigs were treated with musk ambrette. The vehicle used to administer HC Orange No. 1 and

musk ambrette was 80% DAE 433 (40% dimethylacetamide, 30% acetone, and 30% ethanol) and 20% physiologic saline. During the 3-week induction period, 0.1 ml 10% HC Orange No. 1 was applied to a 1.8-cm site on the nuchal area of eight male and eight female guinea pigs for four consecutive days. The animals were irradiated for 7 minutes with 1/2 MED of UVA 1 hour after application (UVB was filtered with a WG-354 glass filter). The skin was scored 24 hours after each application. For the second and third week of induction, the animals received the same treatment with HC Orange No. 1, but were exposed for 90 seconds to 1 MED of UVB light 1 hour after each application. The animals were injected intradermally with 0.1 ml FCA in physiologic saline (1:1) on days 1 and 3 during both weeks. The injections were given at four different sites surrounding the treated area. After a 2-week nontreatment period, each guinea pig was treated with 0.1 ml of 5% HC Orange No. 1 on the left lumbar area for three consecutive days, and was irradiated for 45 seconds with 1/2 MED of UVB 1 hour after each application. The sites were scored after 24 hours. The same procedures were used to test for UVA photosensitization using different sites of application and exposure for 7 minutes to 1/2 MED of UVA. Control sites received the same applications of HC Orange No. 1 but were not exposed to UV light. No evidence of irritation, photosensitization, or contact sensitization to HC Orange No. 1 was observed (CTFA 1986).

Ocular Irritation

The left conjunctival sac of four New Zealand white rabbits was instilled with 100 mg HC Orange No. 1 (concentration not stated). The right eye was untreated and served as controls. The eyes of two rabbits were rinsed after 20 seconds of treatment, while the eyes of the other rabbits were left unrinsed. All of the eyes were examined and scored according to the method of Draize after 1 hour, and 1, 2, and 3 days after treatment. One hour after instillation, all of the treated eyes had conjunctival redness, slight swelling, and discharge. On day 1, the eyes were slightly red, and two of the rabbits had slight conjunctival swelling and ocular discharge. All signs of irritation had disappeared by day 3 (CTFA 1987c).

MUTAGENICITY

HC Orange No. 1 was tested for mutagenic potential in an Ames test at concentrations ranging from 1 to 500 μg/plate both with and without metabolic activation using *Salmonella typhimurium* strains TA98,

TA100, TA1535, TA1537, and TA1538. The positive controls used in this study were 2-aminoanthracene for all strains with metabolic activation; and sodium azide for strains TA100 and TA1535, and 4-nitro-*o*-phenylenediamine for strains TA98, TA1537, and TA1538 without metabolic activation. Dimethylsulfoxide was used as the solvent control for all strains both with and without metabolic activation. At a concentration of 500 μg/plate, HC Orange No. 1 was toxic to the bacteria. At concentrations of 250 μg/plate HC Orange No. 1 and lower, no evidence of a mutagenic response was observed with or without exogenous metabolic activation (Clairol Research & Development Laboratories 1993).

In another Ames test, HC Orange No. 1 was tested at concentrations of 25–5000 μg/plate using *S. typhimurium* strains TA98, TA1537, and TA1538 with metabolic activation. No evidence of mutagenicity was observed, but inhibition or partial inhibition of bacterial growth was observed with concentrations of 100 μg/plate and greater (Fuchs 1991).

HC Orange No. 1 was also tested in an in vitro chromosome aberration study using Chinese hamster ovary (CHO) cells. Cultures of CHO-K1 cells were incubated with 50–300 μg/ml HC Orange No. 1 both with and without S-9 metabolic activation. Positive control cultures were tested with mitomycin C for the nonactivated protocol and with cyclophosphamide for the activated protocol. Concentrations of 150 μg/ml HC Orange No. 1 and greater were toxic to the cells, so the highest dose evaluated was 100 μg/ml. In the nonactivated cultures, HC Orange No. 1 induced a significant and dose-dependent increase in the percentage of metaphase cells with chromatid and chromosome-type breaks and chromatid-type rearrangements. The lowest effective concentration was 100 μg/ml. The mitotic index was also significantly reduced at all of the concentrations tested. In the activated protocol, 50 μg/ml HC Orange No. 1 caused a significant increase in clastogenic damage similar to that observed in the nonactivated protocol. Although not significantly different from concurrent control values, the clastogenic values for concentrations up to 100 μg/ml were of a similar magnitude to that observed with 50 μg/ml, which the investigators suggested was indicative of a saturated clastogenic response. They concluded that HC Orange No. 1 was clastogenic in both the presence and absence of metabolic activation (Integrated Laboratory Systems 1993).

A micronucleus test was conducted in conjunction with the subchronic toxicity and teratogenicity study described earlier in this report. Groups of five male and five female Sprague-Dawley rats fed diets containing 0.125, 0.4, or 1.25% HC Orange No. 1 for 13 weeks were used. A positive control group of rats was fed a diet treated with cyclophosphamide and negative control animals were fed untreated feed. Bone marrow smears were taken and the following parameters were calculated: percent of polychromatic erythrocytes (PCEs) of total erythrocytes, percent

micronucleated PCEs (MN-PCEs) of total PCEs scored, and percent micronucleated normochromatic erythrocytes (MN-NCEs) of total NCEs scored. No evidence of cytotoxicity was observed at any of the doses tested. No significant difference was found in the incidence of MN-PCEs in the bone marrow of the treatment groups as compared to those of the untreated control group. The investigators concluded that HC Orange No. 1 did not have in vivo potential to cause chromosomal or mitotic spindle damage to rat bone marrow cells following subchronic dietary administration (Bristol-Myers Squibb Pharmaceutical Research Institute 1993).

Negative results were also obtained in a rat hepatocyte primary culture and DNA repair test. Triplicate cultures of hepatocytes from Fischer 344 rats were treated with 0.75, 1, 1.5, and 2.5 μg/ml HC Orange No. 1 in dimethylsulfoxide. Positive control cultures were treated with 2-acetamidofluorene, and vehicle control and untreated control cultures were also tested concurrently. HC Orange No. 1 did not increase the mean net nuclear grain count at any of the concentrations tested (Pharmakon Research International, Inc. 1993).

CARCINOGENICITY

A skin painting study was used to assay the carcinogenic potential of a semipermanent hair dye containing 0.15% HC Orange No. 1. Groups of 50 male and 50 female Swiss Webster mice had 0.05 ml of the formulation applied to the clipped interscapular region of their backs. Applications were made once a week for 23 months. Three negative control groups had their coats clipped but did not receive any treatment. The animals were monitored throughout the study for signs of toxicity and necropsy was performed on all of the animals. No treatment-related changes were observed in survival rate or organ-to-body weight ratios. Several neoplasms were found in the treatment group, but the incidence and types of neoplasms were not significantly different from that found in the three control groups (Burnett et al. 1980).

In another study (Goldenthal 1979), groups of 60 male and 60 female Charles River rats were obtained from the first mating (F_{1a}) of a multigeneration reproduction study. The parents (F_0) had been treated topically with a hair dye formulation containing 0.15% HC Orange No. 1 from the time of weaning to the weaning of their offspring. The same hair dye formulation was applied to the shaved neck and back area of the offspring twice a week. The initial dosage was 0.2 ml, which was increased incrementally by 0.1 ml per week until a final dosage level of 0.5 ml was achieved. Three independent control groups of rats were untreated. Observations for signs of toxicity and mortality were

made daily, and body weight and feed consumption were measured regularly. Hematologic, biochemical, and urinalysis studies were conducted with 5 male and 5 female rats from each group at 3, 12, 18, and 24 months. At 12 months, five male and five female rats from each group were killed for necropsy, and all rats were killed for necropsy when survival for that group reached 20%. At week 116, the approximate incidence of palpable masses for the treated rats was 53%. Among the three control groups, the incidence ranged from 54–61%. Eleven of 54 male rats and 15 of 55 female rats in the treatment group survived until the end of the study, whereas 15 of 54 to 15 of 55 male and 14 of 55 to 18 of 56 female rats of the control groups survived. Mean body weights at week 114 were 740 g and 496 g for male and female rats in the treatment group, respectively, whereas the values for the control groups ranged from 682 to 759 g for males and 477 to 513 g for females. Variations in hematology and biochemistry values were isolated. Dark straw-colored urine was observed in the treatment group, with some of the rats eliminating dark brown urine at 12 or 18 months. At necropsy, the only treatment-related findings included skin lesions (ulceration, scabbing, abcessation, and thickening), coloration of the hair and skin at the site of application, and increased incidences of enlarged and/or firm livers. Hyperkeratosis and dermatitis of the skin, hepatocellular hypertrophy or hyperplasia, and parathyroid hyperplasia were also observed and were considered possibly compound related. Pituitary adenoma was the most common neoplasm observed; the incidence of this neoplasm was statistically greater in the treated female rats than in two of the three control groups. However, because there were large variations in the incidence of this neoplasm between the control groups, the investigators discounted any biologic significance to the small increase in the test group. Mammary gland lobular hyperplasia was also significantly increased in treated females as compared to one group of control females, but was not significantly different from the other two controls or all three controls combined. Overall, no significant variations in incidences of tumor bearing animals in the treated group were found when compared to each of the control groups by sex.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In a subchronic toxicity and reproductive and developmental toxicity study described earlier (Bristol-Myers Products 1994), the mid- and high-dose dams weighed significantly less than the controls; however, no evidence of reproductive effects, fetotoxicity, or teratogenicity was observed. An increased number of fetal skeletal variations was observed

in the treated groups, but the incidence was not statistically different from that of the controls.

In a multigeneration study, groups of 40 male and 40 female Charles River CD rats were topically treated with a hair dye formulation containing 0.15% HC Orange No. 1. Applications were made twice a week. An initial dose of 0.2 ml was increased incrementally by 0.1 ml per week until a final dose of 0.5 ml was achieved. Three separate control groups received no treatment. The parental generation (F_0) was treated with the formulation until 100 days of age, when they were mated. The offspring (F_{1a}) were reduced to 10 pups on day 4 of lactation, and some of the pups were used in a lifetime chronic study (see Carcinogenicity section). Twenty F_0 parents of each sex were mated again to produce F_{1b} litters. From this litter, 20 rats of each sex received the same treatment as their parents and were mated twice to produce the F_{2a} and F_{2b} generations. Twenty rats of each sex from the F_{2b} litter were similarly treated and mated to produce the F_{3a}, F_{3b}, and F_{3c} litters. All of the rats were observed regularly for signs of toxicity, and body weight and feed consumption were recorded. Fertility index, gestation anomalies, and effects on parturition and lactation were determined. Pup counts and weights were recorded on days 0, 4, and 14, and individual weights were determined on day 21 of lactation. Live birth and survival indices were also calculated. Necropsy and microscopic examination were conducted on five animals of each sex from the F_1 generation and on one weanling from each litter of the F_{3b} generation. The only treatment-related finding among the parental generations was local skin reactions, including mild scabbing, fissuring, atonia, and leathery texture. No significant changes in fertility, gestation, and live birth indices were found in the F_0, F_1, and F_2 parental rats. Reduced fertility was observed in F_2 parents producing the F_{3a-c} offspring, but no significant differences were found among the groups. No adverse effects on the offspring, in terms of litter size, body weights, and survival, were found, and no treatment-related gross or microscopic lesions were found in the F_1 parents or in the F_{3b} weanlings (Wazeter and Goldenthal 1977).

A semipermanent hair dye formulation containing 0.15% HC Orange No. 1 was cutaneously tested for teratogenic effects using pregnant Charles River CD rats. The backs of 20 rats were shaved and 2 ml/kg of the formulation was applied to these sites 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. No significant changes in soft-tissue anomalies or skeletal variations were observed

between the fetuses of the treatment group and the negative controls. An increase in embryotoxicity was seen in positive controls, reportedly consistent with other studies of the effect of aspirin on fetal rat development (Burnett et al. 1976).

CLINICAL ASSESSMENT OF SAFETY

Sensitization

The sensitization potential of HC Orange No. 1 was tested using a repeated insult patch test. Ninety-eight individuals (13 male and 85 female; age range from 18 to 78; primarily Caucasian) had 0.15 ml 3% HC Orange No. 1 applied under occlusive patches to the infrascapular region of their backs. The subjects removed the patches after 24 hours. The treated sites were scored at 48 hours and new patches were applied. Nine consecutive applications were made during the induction phase. Following a 14-day nontreatment period, patches of HC Orange No. 1 were applied to previously unexposed sites. The patches were removed after 24 hours, and the sites were graded 24 and 48 hours later. A small number (1–3) of subjects had erythema during the induction phase of the study, and there was no evidence of sensitization caused by HC Orange No. 1 (TKL Research, Inc. 1985a).

Following the same procedures outlined above, another repeat insult patch test was conducted using 101 subjects (15 male and 86 female; age range from 18 to 79; primarily caucasian). During the induction phase, the number of questionable reactions to HC Orange No. 1 was large, ranging from 6 to 53 reactions. The investigators believed that the incidence of irritation was much less than the data indicated because the dye caused epidermal staining. A few cases of erythema were also observed during the induction phase. Only one subject had a reaction during the challenge phase; erythema was present at 48 hours but disappeared by 72 hours (TKL Research, Inc. 1985b).

EPIDEMIOLOGY

Between 35% and 45% of American women dye their hair, often at monthly intervals, over a period of years (CTFA 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 to individuals using hair dyes is unclear.

SUMMARY

HC Orange No. 1 is used as a colorant in semipermanent hair dyes at reported concentrations of up to 0.15%. Data submitted to the FDA in 1996 indicated that this ingredient was used in a total of 95 hair dyes and colors. Hair dyes containing HC Orange No. 1, as coal tar hair dyes, are exempt from the principal adulteration provision 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.

In vitro skin penetration data indicated that following 30 minutes of exposure, the cumulative absorption of a hair dye containing 1.0% HC Orange No. 1 through human cadaver skin was 1.28% at 24 hours. The permeation rate peaked at 1.5 hours and rapidly decreased to a steady level 6 hours later.

The oral LD_{50} for 10% HC Orange No. 1 was >5000 mg/kg for male rats and >1250 mg/kg for female rats. In a short-term toxicity study, rats fed diets containing concentrations of 0.5% HC Orange No. 1 and greater had significantly lower body weights. Relative liver weights were increased in male rats fed 0.875% HC Orange No. 1 and in both male and female rats fed 1.25%. Relative kidney weights were also increased in female rats fed the 1.25% diet.

In a combined subchronic oral toxicity and teratogenicity study in which male and female rats were fed 0.125, 0.4, and 1.25% HC Orange No. 1 in the diet, reduced body weights were observed in both

sexes. Dose-related microscopic lesions were found in the kidneys, and, in male rats, hyaline droplets in the proximal convoluted tubule were observed. Thyroid follicular cell hypertrophy was observed in all of the treated male rats and in the mid- and high-dose female rats. Despite low maternal body weights, no evidence of reproductive or teratogenic effects was found.

In a multigeneration study, a hair dye formulation containing 0.15% HC Orange No. 1 was topically applied to parental rats through three generations. No effects on fertility, gestation, live birth indices, litter size, offspring body weight, or survival were observed.

Ocular studies indicated that HC Orange No. 1 was a mild irritant to the eyes of rabbits. HC Orange No. 1 was not a primary irritant to the skin of rabbits, and there was no evidence from animal studies that it was either a sensitizer or photosensitizer. Negative results were also obtained in clinical sensitization studies.

HC Orange No. 1 was negative for mutagenicity in Ames microbial assays, a bone marrow erythrocyte micronucleus assay in rats, and an unscheduled DNA repair test in rat hepatocyte cells. However, HC Orange No. 1 was positive for clastogenicity in a chromosome aberration study with CHO cells both with and without metabolic activation.

There was no indication of carcinogenicity of semipermanent hair dye formulations containing 0.15% HC Orange No. 1 in long-term dermal studies with mice and rats.

DISCUSSION

After assessing the available safety test data, the CIR Expert Panel considered that HC Orange No. 1 could safely be used in hair dyes. The question remained: at what concentrations?

This ingredient was reported to be used at concentrations up to 0.15% by one manufacturer, but that manufacturer could not be certain if higher concentrations may be used by others. Additionally, the possibility that concentrations could increase in the future was raised. Faced with that uncertainty about the concentrations in current use, the Panel concluded that the available data could support a finding of safety only up to the concentrations actually tested. In this regard, the negative dermal carcinogenicity study using a concentration of 0.15% and the absence of significant sensitization in clinical studies using a concentration of 3.0% were of importance.

Because the carcinogenicity study was negative, the Expert Panel initially viewed the most significant potential adverse effect as dermal irritation and sensitization. Based on the available data, the Expert Panel reached a tentative conclusion at its March 16–17, 1995, meeting that HC Orange No. 1 would be safe for use in hair dyes at concentrations

no greater than 3%. No public comment was received on this tentative conclusion.

At its August 28–29, 1995, meeting, the Expert Panel reviewed its previous tentative conclusion and revised it. Focusing on data indicating that HC Orange No. 1 did produce chromosome aberrations in a mammalian system genotoxicity test and hyperplastic changes in certain tissues in the dermal carcinogenicity study, the Expert Panel decided that safety could only be assured up to the level actually tested in the dermal carcinogenicity study (0.15%). Accordingly, a revised tentative conclusion—safe for use in hair dyes at concentrations no greater than 0.15%—was offered for public comment.

During the public comment period, the Expert Panel was requested to consider a conclusion that this ingredient is safe for use in hair dyes at concentrations of at least 1% (Clairol 1996). In support of this position, the comment analyzed the possible human exposure levels that took into consideration the concentration of the ingredient in hair dyes, the quantity applied, the duration of exposure, the very slow absorption of the ingredient, and the available toxicity data. Based on this analysis, it was suggested that HC Orange No. 1 could be used at concentrations $\leq$3% in hair dyes and not actually result in skin exposures as great as the 0.15% used in the long-term dermal carcinogenicity study. It was argued that this ingredient would not present carcinogenic or any other risk if the Panel returned to its original conclusion.

In its further deliberations, the CIR Expert Panel noted that the preponderance of data (four out of five studies) indicate that this ingredient is not genotoxic. And while hepatocellular and parathyroid hyperplasia was noted in the dermal carcinogenicity study, the overall findings were clearly negative. Additionally, oral and topical application of HC Orange No. 1 did not result in reproductive or developmental toxicity. These data, along with the exposure analysis above, and the evidence that HC Orange No. 1 is poorly absorbed through the skin, were sufficient for the Expert Panel to conclude that adverse systemic effects from the use of cosmetic products containing this ingredient would be highly unlikely. Because the highest concentration tested that produced no significant sensitization was 3%, the Expert Panel concluded that safety in terms of this endpoint could be assured only at levels $\leq$3%. The Expert Panel recognizes that this concentration may be greater than that currently used in hair dye formulations.

CONCLUSION

On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that HC Orange No. 1 is safe for use in hair dye formulations at concentrations up to 3.0%.

REFERENCES

Adams, R. M., H. I. Maibach, W. E. Clendenning, et al. 1985. A five-year study of cosmetic reactions. *J. Am. Acad. Dermatol.* 13:1062–1069.

Bristol-Myers Products. 1994. The toxicity of 2-nitro-4'-hydroxydiphenylamine (HC Orange No. 1) in the Sprague-Dawley rat: Effects on growth, reproduction, and fetal development. Study No.: GLP-90027. Unpublished data submitted by Clairol, Stamford, CT. 33 pages.*

Bristol-Myers Squibb Pharmaceutical Research Institute. 1993. Dietary bone marrow erythrocyte micronucleus evaluation in rats. Study No.: GLP-90027. Unpublished data submitted by Clairol, Stamford, CT., 18 pages.*

Burnett, C., M. M. Jacobs, A. Seppala, and P. Shubik. 1980. Evaluation of the toxicity and carcinogenicity of hair dyes. *J. Toxicol. Environ. Health* 6:247–257.

Burnett, C., E. I. Goldenthal, S. B. Harris, et al. (1976) Teratology and percutaneous toxicity studies on hair dyes. *J. Toxicol. Environ. Health* 1:1027–2040.

Clairol. 1995. Types and numbers of products containing HC Orange No. 1 and manufacturing data. Unpublished data submitted by Clairol, Stamford, CT., 1 page.*

Clairol. 1996. Percutaneous absorption (*in vitro/in vivo* comparisons) and a rationale to support the use of HC Orange No 1 in hair dye formulations at levels of 1.0% or higher. Unpublished data submitted by Clairol, 17 pages.*

Clairol Research & Development Laboratories. 1993. Ames microbial assay with HC Orange No. 1. Study No.: GLP-92050. Unpublished data submitted by Clairol, Stamford, CT., 56 pages.*

Clairol Research & Development Laboratories. 1994. *In vitro* skin penetration of ^{14}C-HC Orange No. 1. Study No.: 94017. Unpublished submitted by Clairol, Stamford, CT., 26 pages.*

Cosmetic, Toiletry, and Fragrance Association (CTFA). 1986. Photosensitization potential of H. C. Orange #1 in guinea pigs. Study No.: 86020. Unpublished data submitted by CTFA, 11 pages.*

CTFA. 1987a. Acute oral toxicity (C6674-002). Unpublished data submitted by CTFA, 2 pages.*

CTFA. 1987b. Dermal irritation (C6675-006). Unpublished data submitted by CTFA, 2 pages.*

CTFA. 1987c. Ocular irritation (C6676-018). Unpublished data submitted by CTFA, 2 pages.*

CTFA. 1992. Method of manufacture and purity. Unpublished data submitted by CTFA, 1 page.*

CTFA. 1993. Personal correspondence from GN McEwen dated 12/06/93, 2 pages.*

Eiermann, H. J., W. Larsen, H. I. Maibach, et al. 1982. Prospective study of cosmetic reactions: 1977–1980. *J. Am. Acad. Dermatol.* 6:909–917.

Elder, R. L. ed. 1985. Final report on the safety assessment of p-phenylenediamine. *J. Am. Coll. Toxicol.* 4:203–266.

*Available for review from the Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

Food and Drug Administration (FDA). 1979. Cosmetic product warning statements; coal tar hair dyes containing 4-methoxy-*m*-phenylenediamine (2,4-diaminoanisole) or 4-methoxy-*m*-phenylenediamine sulfate (2,4-diaminoanisole sulfate). *Federal Register* 44:59509–59510.

FDA. 1992. Modification in voluntary filing of cosmetic product ingredient and cosmetic raw material composition statements. Final rule. *Federal Register* 57:3128–3130.

FDA. 1996. Frequency of use of cosmetic ingredients. FDA database. Washington, DC: FDA.

Fuchs, C. 1991. Mutagenic testing results. Data from studies initiated January 13, 1977 and March 8, 1977. Clairol report dated January 9. Unpublished data submitted by Clairol, Stamford, CT., 1 page.*

Goldenthal, E. I. 1979. Lifetime toxicity/carcinogenesis study in rats. Report to Clairol 355-033b, International Research and Development Corporation. Report dated April 10. Unpublished data submitted by Clairol, Stamford, CT., 4 pages.*

Integrated Laboratory Systems. 1993. *In vitro* chromosome aberrations study in Chinese hamster ovary (CHO) cells. Clairol Study No. GLP-92052. Unpublished data submitted by Clairol, Stamford, CT., 29 pages.*

International Agency for Research on Cancer (IARC). 1993. *IARC monographs on the evaluation of carcinogenic risks to humans*. Vol. 57, 43–118. Lyon, France: IARC.

Loehr, R. 1979. Delayed contact sensitization in the albino guinea pig with HC Orange No. 1. Clairol Report No. C4206-29, dated March 30. Unpublished data submitted by Clairol, Stamford, CT., 1 page.*

Loehr, R., and T. A. Re. 1990. Four week feeding study on HC Orange 1 in Sprague-Dawley rats. Clairol Report 90021 dated July 10. Unpublished data submitted by Clairol, Stamford, CT., 1 page.*

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

Pharmakon Research International, Inc. 1993. Rat hepatocyte primary culture/DNA repair test on C7634/71. PH 311-CA-003-92. Unpublished data submitted by Clairol, Stamford, CT., 87 pages.*

TKL Research, Inc. 1985a. Repeated insult patch test. Study #841021. Unpublished data submitted from CTFA, 27 pages.*

TKL Research, Inc. 1985b. Repeated insult patch test. Study #851002. Unpublished data submitted from CTFA, 30 pages.

Wazeter, F. X., and E. I. Goldenthal. 1977. Multi-generation reproduction study in rats. Report to Clairol 355-002b, International Research Development Corporation. Report dated November 4. Unpublished data submitted by Clairol, Stamford, CT., 2 pages.*

Wenninger, J. A., G. N., McEwen, Jr., eds. (1997) *International cosmetic ingredient dictionary and handbook*. 7th ed., Vol. 1, 593. Washington, DC: CTFA.