Journal of the American College of Toxicology
13(3):157–166, Raven Press, Ltd., New York

Final Report on the Safety Assessment of HC Yellow No. 2

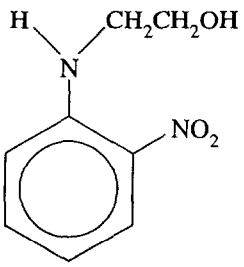
Summary: HC Yellow No. 2 is used in oxidative or semipermanent hair dye formulations in concentrations up to 1%. The LD_{50} for HC Yellow No. 2 administered via gavage was between 1.2 and 2.5 g/kg in male rats and between 0.6 and 1.2 g/kg in female rats. In the subchronic and chronic feeding studies, 1.25% HC Yellow No. 2 reduced body weight gain and induced changes in various organ sizes and clinical chemistry values. The only histologic change attributed to this hair dye was a small increase in the pigment of the spleen. The compound was only a minor ocular irritant when tested at a concentration of 10%. It was neither a sensitizer nor photosensitizing agent in guinea pigs at 10%. It was not a teratogenic compound in rats. HC Yellow No. 2 was negative in a dominant lethal assay and it was not mutagenic in the four *S. typhimurium* strains tested, both with or without S9 metabolic activation. In a repeated insult patch test (RIPT) using 3% HC Yellow No. 2, two volunteers of 98 had a positive reaction to the test substance. In another RIPT study, 1 of 104 volunteers had a sensitization reaction to 3% HC Yellow No. 2. It is concluded that HC Yellow No. 2 is safe for use in hair dyes at concentrations up to 3%.
Key Words: Safety assessment—HC Yellow No. 2.

The following is a summary of data available to Cosmetic Ingredient Review (CIR) concerning the chemistry, toxicity, and cosmetic use of HC Yellow No. 2.

CHEMISTRY

Definition and Structure

HC Yellow No. 2 (CAS No. 4926-55-0) is the aromatic compound that conforms to the following formula:



Other names for HC Yellow No. 2 include ethanol, 2-[(2-nitrophenyl)amino; *N*-(2-hydroxyethyl)-2-nitroaniline; 2-[(2-nitrophenyl)amino]ethanol (Estrin et al., 1982).

USE

The only reported use of HC Yellow No. 2 is in hair dyes/colors and hair rinses. Data submitted to the Food and Drug Administration in 1984 by cosmetic firms participating in the voluntary cosmetic registration program indicated that HC Yellow No. 2 was used in a total of 57 hair dyes and colors at a maximum concentration of 1% (FDA, 1984). In 1992, it was used in 91 hair dye formulations (Table 1) requiring a cautionary statement (FDA, 1992).

Hair-coloring formulations containing HC Yellow 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 Yellow 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 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), although the oxidation and subsequent coupling reactions go to nearly 100% completion, leaving little if any original dye precursor material.

The oxidative or permanent hair dyes containing the HC Yellow No. 2, as "coal tar" hair dye products (Elder, 1985*a*), 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 eyebrows; to do so may cause blindness.

At one time, patch test instructions called 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 was to be performed before each and every application of the hair dye (Corbett and Menkart, 1973).

TABLE 1. *Product formulation data (FDA, 1992)*

Product category	Total no. containing ingredient
Hair dyes/colors (requiring a cautionary statement)	91
1992 Total	91

However, 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 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 (NACDG) and the International Contact Dermatitis Group (ICDG).[1,2,3] 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 NACDG and the ICDG in its safety evaluation reports on cosmetic ingredients.[4]
>
> 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.

ANIMAL TOXICITY

Oral

Acute

The approximate LD_{50} of HC Yellow No. 2, administered via gavage in 3% acacia, was between 1,250 and 2,500 mg/kg in male rats and between 625 and 1,250 mg/kg in female rats (CTFA, 1991).

Subchronic

The subchronic toxicity of HC Yellow No. 2 was studied in Sprague–Dawley rats (CTFA, 1991*a*). There were four treatment groups: Group A, comprising 40

---

[1] North American Contact Dermatitis Group. (1980) Patch testing in allergic contact dermatitis. Evanston, IL: American Academy of Dermatology.

[2] Eierman et al. (1982) Prospective study of cosmetic reactions: 1977–1980. *J Am Acad Dermatol* 6:909–17.

[3] Adams et al. (1985) A five-year study of cosmetic reactions. *J Am Acad Dermatol* 12:1062–9.

[4] Elder, ed. (1985*a*) Final report on the safety assessment of *p*-phenylenediamine. *J Am Coll Toxicol* 43:203–66.

males and 45 females, received a control diet; Group B, 40 males and 45 females, received 0.125% HC Yellow No. 2 in their feed; Group C, 40 males and 55 females, received 0.40% HC Yellow No. 2 in their feed; and Group D, 45 males and 55 females, received 1.25% HC Yellow No. 2. Animals were given feed and water ad libitum. Animals were examined and weighed weekly. After week 6, blood was taken from the retro-orbital sinus of five males and five females from the control and high-dose groups, as well as five males and five females that had been treated with phenacetin. The blood samples were assayed for methemoglobin concentrations. After week 13, 10 males and 10 females from each group were selected, fasted for 24 h, and then killed for necropsy. Hematologic and clinical chemistry assays were performed on the blood.

Group D males and females had significantly decreased feed consumption and weight gain during most of the study as compared with controls. Groups B and C had significantly decreased feed consumption and weight gain sporadically during the study as compared with controls.

Group D males had a significant increase of methemoglobin concentration compared with controls, but lower than the phenacetin-treated animals by 15-fold. No significant variations in hematologic values (serum or whole blood not stated) for either sex were observed between treated and control groups. Group B females had significant increases in total protein, albumin, and calcium. Group C females had significant increases in total protein, albumin, Albumin/Globulin (A/G) ratio, cholesterol, and calcium. Group D females had significant increases in total protein, albumin, A/G ratio, cholesterol, and calcium. Group D males had a significant increase in cholesterol.

Absolute and relative weights of the liver were increased significantly in dosed groups with the exception of Group B females. Group D males also had significantly increased relative weights of the brain. Females in Groups C and D had significant increases in absolute and relative weights of the thyroid gland.

There were no significant histopathologic changes attributable to HC Yellow No. 2 (CTFA, 1991).

Chronic

Sprague–Dawley rats from the previous subchronic toxicity study were used in a chronic feeding study (CTFA, 1991*a*). Ten rats of each sex per group were maintained on their initial diets for an additional 90 days (for a total of 6 months). Also, 10 females from Groups C and D were fed HC Yellow No. 2 for a total of 23 weeks, and then given basal chow for the remainder of the study. Additionally, 20 males per group from the dominant lethal study (see Genotoxicity section) were maintained on basal chow until the end of the study. These last two sets of animals were termed "recovery animals." Animals were maintained, killed, underwent necropsy and were evaluated as they were in the subchronic study.

Group D males and females had sporadic significant decreases in mean body weight gain. Some animals in Groups C and D had discoloration of the fur. Some males in Group D had discolored thyroid glands.

Group D males had increased absolute weights of the kidneys, brain, testes,

thyroid glands, and liver as compared with controls. Group C males had increased absolute weights of the liver. Group D females had increased relative weights of the liver, adrenal glands, heart, and kidneys and absolute weights of the liver. Group C females had increased absolute and relative weights of the liver and relative weights of the kidneys. Group B females had increased absolute and relative weights of the liver.

No significant differences between dosed and control groups were found in hematologic values.

Group D males had significant increases in albumin and decreases in activities of aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) as compared with the control. Groups B and C males had decreased activities of LDH. Group D females had increases in albumin, calcium, total protein, and cholesterol values as compared with the control. Group C females had increases in albumin, total protein, and cholesterol values. Group B females had increases in albumin, calcium, and glucose values.

In the "recovery animals," Group D male and female body weights were sporadically decreased. Group B males had a significant decrease in absolute and relative weights of the adrenal glands. Group C females had a decrease in absolute weights of the ovaries. Group D females had a decrease in absolute weight of the adrenal glands.

The only histomorphic change attributed to the HC Yellow No. 2 was a small increase in pigment in the spleen.

Ocular Irritation

HC Yellow No. 2, 100 mg in powder form, was instilled into the left conjunctival sac of four New Zealand White rabbits. The right eyes were untreated. In two of the rabbits, the eyes were rinsed with 20 ml of distilled water 20 s after instillation. Animals had conjunctival redness, swelling, and ocular discharge in treated eyes after 1 h. After 1 day, three animals had slight corneal opacity with ulcerations, and one animal had iritis. No signs of ocular irritation were observed after 3 days. Eyes rinsed with distilled water had less irritation than those that were not rinsed (CTFA, unpublished data, 1991*a*).

The same study was conducted using 0.1 ml of 10% HC Yellow No. 2 in 3% acacia. Minor conjunctival irritation was noted in dosed eyes, both rinsed and unrinsed, after 1 h. All eyes were clinically normal after 2 days (CTFA, 1991*a*).

Dermal

Primary Irritation

HC Yellow No. 2, 500 mg as an aqueous slurry, was applied to the backs of six New Zealand White rabbits and left in open contact with the skin for 24 h. Sites were scored 24 and 72 h postapplication. No dermal irritation was produced by HC Yellow No. 2 (CTFA, 1991*a*).

Sensitization and Photosensitization

The photosensitization potential of HC Yellow No. 2 was studied using Hartley albino guinea pigs. For 4 consecutive days during the first week of induction, 0.1 ml of 10% HC Yellow No. 2 in acacia was applied to a 1.8-cm diameter shaved and depilated nuchal area of eight female and eight male guinea pigs. After 1 h, animals were irradiated with a 150 W Xenon lamp with a WG-354 glass filter for 7 min, an exposure equal to one half the minimal erythemal dose (MED) for UVA light in guinea pigs. During the second and third week of induction, HC Yellow 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, an exposure 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 5% HC Yellow 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 above). No irritation was observed during the induction phase of the study. HC Yellow No. 2 did not induce any sensitization or photosensitization reactions. All of the positive control animals had photosensitization reactions (CTFA, 1991*a*).

REPRODUCTIVE

Teratology

Female Sprague–Dawley rats from the previously summarized subchronic feeding study (CTFA, 1991*a*) were included in a teratology study of HC Yellow No. 2. Twenty-five females from Groups A–D were maintained on their test diets until mating. Animals were changed to basal laboratory chow during mating. On the day determined to be gestation day 0, animals were returned to the appropriate test diet. Females were killed on day 20 of gestation and fetuses were removed. Gravid uterine weight and the number of live fetuses, dead fetuses, and resorptions were determined. Fetuses were examined for sex and gross malformations. Dead fetuses were stored in 10% neutral buffered formalin. Of the live fetuses, some were stored in Bouin's solution and some were stored in alcohol. Those stored in alcohol were later cleared in 2% KOH, stained with Alizarin Red-S and put in glycerin. A significant decrease in maternal mean body weight gain was observed in Group D as compared with the control. No significant differences were observed in average litter or reproduction data between dosed and control groups. No malformations in the fetuses were attributable to HC Yellow No. 2.

GENOTOXICITY

Male Sprague–Dawley rats from the previously summarized subchronic study (CTFA, 1991*a*) were selected for a dominant lethal study. Twenty males from each group were removed from their test diets and immediately mated with untreated females, two per male. Males were mated with two new females 1 week

later. Males remained on control diets for 8–9 weeks, when they were killed. Pregnant females were killed on day 17 of gestation. Nonpregnant females were killed 17 days from the midpoint of the mating period. Necropsy was performed and live fetuses, dead fetuses, and resorptions were counted. No significant differences in reproduction parameters were observed between the treated and control groups.

HC Yellow No. 2 was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 in concentrations up to 5,000 μg/plate with or without S9 metabolic activation (CTFA, 1991*b*).

CLINICAL ASSESSMENT OF SAFETY

Sensitization

A repeated insult patch test (RIPT) on various materials including HC Yellow No. 2 was completed using 98 volunteers (CTFA, 1991*a*). During the induction phase, HC Yellow No. 2, 3% in acacia, was applied under an occlusive patch to intact skin every 48 h for a total of 10 applications. Sites were scored before each new application. An 11-day nontreatment period followed. The challenge phase consisted of a single 48-h patch. Sites were graded immediately and 24 h after removal of the patch. Two panelists had slight erythematous reactions to the challenge patch, but after further testing, the reactions were considered due to irritation and not sensitization.

Another RIPT, performed exactly as the previous study, used a different group of 104 volunteers (CTPA, 1991*a*). One volunteer developed definite erythema and edema during both induction and challenge phases of the study. This reaction was considered due to a presensitization to the vehicle.

EPIDEMIOLOGY

Approximately 40% of American women dye their hair, often at monthly intervals over a period of years (Corbett and Menkart, 1973). The U.S. Environmental Protection Agency (EPA) (1982) reported that ~15 million people are potentially exposed to hair dye ingredients as a result of personal use or in the application of hair dyes to other people.

A variety of published studies have assessed the association between occupational exposure to and use of hair dyes and the risk of cancer. These studies do not note which specific hair dye ingredients were involved in the human exposure. Summaries of reports on how occupational exposure to hair dye affects the risk of bladder cancer (Cole et al., 1972; Anthony and Thomas, 1970; Dunham et al., 1968; Wynder et al., 1963) and lung cancer (Garfinkel et al., 1977; Menck et al., 1977), and how the personal use of hair dyes affects the risk of bladder cancer (Jain et al., 1977) and breast cancer in women (Wynder and Goodman, 1983; Hennekens et al., 1979; Shore et al., 1979; Nasca et al., 1979; Kinlen et al., 1977; Shafer and Shafer 1976) have been published in previous CIR reports on *p*-phenylenediamine, 2-nitro-*p*-phenylenediamine, and 4-nitro-*o*-phenylenediamine (Elder 1985*a*,*b*).

In the small case-controlled study by Shore et al. (1979), a positive correlation between hair dye and breast cancer was reported. When their study was extended to include 398 breast cancer cases, the same investigators could not implicate hair dye use as an important cause of human breast cancer (Koenig et al., 1991). The latter study indicated that beauticians who work for 5 or more years in this occupation have an increased breast cancer risk. However, the increased risk was not a strong finding, and "if beauticians are at increased breast cancer risk, exposures other than hair dyes may be responsible" (Koenig et al., 1991).

An epidemiologic prospective study involving 118,404 U.S. women concluded that the use of permanent hair dyes appears unlikely to cause any important increase in the risk of breast cancer (Green et al., 1987).

There has been insufficient evidence of any carcinogenic effect from hair dyes on the organs investigated among the occupations and users examined (Clemmesen, 1981). Clemmesen (1981) discussed the difficulties implicit in epidemiologic studies and reviewed many of the papers that investigated the relation of the risk of cancer to occupational exposure to or use of hair dyes. He concluded that most researchers used samples that were too small to allow conclusions and that analyses of duration and intensity of exposure, lag time, and the influence of lifestyle factors, such as tobacco use, were deficient in many cases.

SUMMARY

HC Yellow No. 2 is used in a total of 91 oxidative or semipermanent hair dye formulations in concentrations up to 1%. The oxidative or permanent hair dyes containing HC Yellow 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 appropriate "patch test" instructions for determining whether the product causes skin irritation. The patch test, in which the intermediates and hydrogen peroxide are mixed in the same manner as in use, is to be performed before each and every application of the hair dye.

The LD_{50} for HC Yellow No. 2 was between 1,250 and 2,500 mg/kg in male rats and between 625 and 1,250 mg/kg in female rats. In the subchronic and chronic dosed feed studies, 1.25% HC Yellow No. 2 reduced body weight gain and induced changes in various organ sizes and clinical chemistry values.

The compound is a minor ocular irritant at 10%. It is not a primary skin irritant. It is neither a sensitizer nor photosensitizing agent in guinea pigs at 10%. It is not teratogenic.

HC Yellow No. 2 was negative in a dominant lethal assay. It was not mutagenic in the four *S. typhimurium* strains tested with or without S9 metabolic activation.

In a RIPT using 3% HC Yellow No. 2, two of 98 volunteers had a positive reaction to the test substance. In another RIPT study, 1 of 104 volunteers had a sensitization reaction to 3% HC Yellow No. 2.

DISCUSSION

The CIR Expert Panel recognizes that concentration of use data are no longer submitted to the FDA by the cosmetics industry. Due to this fact, the Expert

Panel can no longer make the conclusion "Safe as used," as was previously done, but must now make a conclusion based on the product and test concentrations used in the report.

In their review of HC Yellow No. 2, the CIR Expert Panel considered the absence of significant toxicity at concentrations tested in the available studies. The highest dose of HC Yellow No. 2 in the oral subchronic study was 1.25%. The highest dose in the animal sensitization study, 10% HC Yellow No. 2, produced no effects. Of more consequence to the Panel, however, was the lack of significant adverse effects in a clinical population at 3%.

CONCLUSION

The CIR Expert Panel concludes that HC Yellow No. 2 is safe for use in hair dyes at concentrations up to 3%. The limitation on the concentration is based upon the available clinical sensitization data.

Acknowledgment: Lynn Willis, Scientific Analyst and Writer, prepared this report.

REFERENCES

Anthony HM, Thomas GM. (1970) Tumors of the urinary bladder: An analysis of the occupations of 1,030 patients in Leeds, England. *J Natl Cancer Inst* 45:879–98.

Clemmesen J. (1981) Epidemiological studies into the possible carcinogenicity of hair dyes. *Mutat Res* 87:65–79.

Cole P, Hoover R, Friedall CH. (1972) Occupation and cancer of the lower urinary tract. *Cancer* 29:1250–60.

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

Cosmetic, Toiletry, and Fragrance Association (CTPA). (1991*a*) Submission of unpublished data: Acute oral, acute dermal, ocular irritating photosensitization, two RIPT studies, and a combined subchronic, chronic, teratology, and dominant lethal study. Received June 5, 1991. (255 pp.)*

CTPA. (1991*b*). Submission of unpublished data: Mutagenicity studies. Received June 17, 1992. (255 pp.)*

Dunham LJ, Rabson AS, Stewart HL, Frank AS, Young JL. (1968) Rate, interview, and pathology study of cancer of the urinary bladder in New Orleans, LA. *J Natl Cancer Inst* 41:683–709.

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

Elder RL, ed. (1985*b*) Final report on the safety assessment of 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine. *J Am Coll Toxicol* 4:161–202.

Environmental Protection Agency (EPA). (1982) Phenylenediamines: response to interagency testing committee. *Fed Register* 47:979.

Estrin NF, Crosley PA, Haynes CR, eds. (1982) *CTFA Cosmetic Ingredient Dictionary*, 3rd ed. Washington, DC: The Cosmetic Toiletry and Fragrance Assoc. 119.

Federal Register. (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). 44:59509–10.

Food and Drug Administration (FDA). (1984) Cosmetic product formulation data. Washington, DC.

FDA. (1992) Cosmetic product formulation data. Washington, DC.

Garfinkel J, Selvin S, Brown SM. (1977) Possible increased risk of lung cancer among beauticians. *J Natl Cancer Inst* 58:141–3.

Green A, Willet WC, Colditz GA, et al. (1987) Use of permanent hair dyes and risk of breast cancer. *J Natl Cancer Inst* 79:253–7.

* Available for review—Director Cosmetic Ingredient Review, 1101 17th Street, N.W., Suite 310, Washington, DC 20036.

Hennekens CH, Speizer FE, Rosner B, Bain CJ, Belanger C, Peto R. (1979) Use of permanent hair dyes and cancer among registered nurses. *Lancet* 1:1390–3.
Jain M, Morgan RW, Elinson L. (1979) Hair dyes and bladder cancer. *Can Med Assoc J* 117:1131–3.
Kinlen LJ, Harris R, Garrod A, Rodriguez K. (1977) Use of hair dyes by patients with breast cancer: a case-control study. *Br Med J* 2:366–8.
Koenig K, Pasternack BS, Shore RE, Strat P. (1991) Hair dye use and breast cancer: a case-controlled study among screening participants. *Am J Epidemiol* 133:985–95.
Menck HR, Pike MC, Henderson BE, Jing JS. (1977) Lung cancer risk among beauticians and other female workers. *J Natl Cancer Inst* 59:1423–5.
Nasca PC, Lawrence CE, Greenwald P, Chorost S, Arbuckle JT, Paulson A. (1979) Relationship of hair dye use, benign breast disease, and breast cancer. *J Natl Cancer Inst* 64:23–8.
Shafer N, Shafer RW. (1976) Potential of carcinogenic effects of hair dyes. *NY State J Med* 76:394–6.
Shore RE, Pasternack BS, Thiessen EU, Sadow M, Forbes R, Albert RE. (1979) A case-control study of hair dye use and breast cancer. *J Natl Cancer Inst* 62:277–83.
Wynder EL, Goodman M. (1983) Epidemiology of breast cancer and hair dyes. *J Natl Cancer Inst* 71:481–8.
Wynder EL, Onderdonk J, Mantel N. (1963) An epidemiological investigation of cancer of the bladder. *Cancer* 16:1388–407.