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Final Report on the Safety Assessment of 2,4-Diaminophenol and 2,4-Diaminophenol Dihydrochloride[1]

Abstract: The aromatic amines 2,4-Diaminophenol and 2,4-Diaminophenol Dihydrochloride are colorants intended for use in oxidative hair dyes. Currently, 2,4-Diaminophenol Dihydrochloride is used in three hair dyes, whereas there are no reported uses of 2,4-Diaminophenol. Chromatographic analysis of 2,4-Diaminophenol indicates that an impurity, 2-amino-4-nitrophenol, is present at a concentration of 2.7%. The oral median lethal dose (LD_{50}) for 2,4-Diaminophenol Dihydrochloride in rats was 0.24 g/kg, with a dose-dependent renal toxicity seen in acute, short-term, subchronic and chronic toxicity tests. Renal toxicity was noted in rats at doses nine times lower than the LD_{50}. 2,4-Diaminophenol Dihydrochloride was a skin and ocular irritant in rabbits, but not in guinea pigs, nor did it induce sensitization in guinea pigs. A dye formulation with 0.2% 2,4-Diaminophenol did not produce evidence of teratogenesis in female rats. No genotoxicity was seen in some systems, but there was evidence of mutagenesis in others. Dermal application of 2,4-Diaminophenol was not carcinogenic in Swiss Webster mice in a 23-month study, even with the presence of a 2.7% 2-amino-4-nitrophenol impurity. Administration of 2,4-Diaminophenol Dihydrochloride by gavage produced no evidence of carcinogenic activity in National Toxicology Program bioassays in male and female F344/N rats and female $B6C3F_1$ mice. There was an increase in renal tubular cell adenomas in male $B6C3F_1$ mice at the highest dose level, 38 mg/kg. On the basis of the animal data presented in the report, the expected use of the product, and acknowledging the labeling requirements for hair dyes if they are to qualify for an exemption from the Food and Drug Administration, it is concluded that 2,4-Diaminophenol and 2,4-Diaminophenol Dihydrochloride are safe for use in hair dyes at concentrations up to 0.2% (as the free base).
Key Words: 2,4-Diaminophenol—2,4-Diaminophenol Dihydrochloride—Safety—Cosmetic use—Hair colorant—Rat—Guinea pig—Mouse—Human—Chemistry—Toxicity—Mutagenicity—Carcinogenicity.

2,4-Diaminophenol and 2,4-Diaminophenol Dihydrochloride are hair colorants used exclusively in oxidative hair dyes. The following is a summary of the data available to the Cosmetic Ingredient Review (CIR) concerning the chemistry, cosmetic use, general and developmental toxicity, mutagenicity, and carcinogenicity of these compounds.

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

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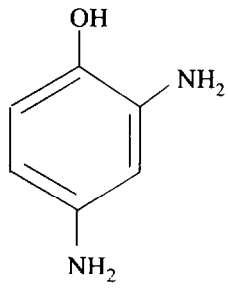
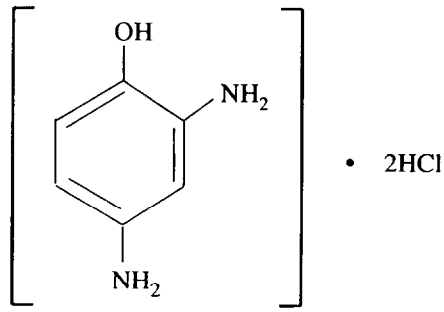
CHEMISTRY

Definition and Structure

2,4-Diaminophenol (CAS No. 95-86-3) and 2,4-Diaminophenol Dihydrochloride (CAS No. 137-09-7) are aromatic amines that conform to the formulae:



2,4-Diaminophenol
(Nikitakis et al., 1991)



2,4-Diaminophenol Dihydrochloride
[National Toxicology Program (NTP), 1992]

Other names for 2,4-Diaminophenol Dihydrochloride include acrol, amidol, and dianol (Windholz et al., 1983; NTP, 1992).

Chemical and Physical Properties

2,4-Diaminophenol, molecular weight 124.14, occurs as crystals that decompose at 78–80°C. It is very soluble in acids and alkalies, somewhat soluble in alcohol and acetone, and slightly soluble in ether, chloroform, and petroleum ether (Windholz et al., 1983). 2,4-Diaminophenol Dihydrochloride, molecular weight 197.07, also occurs as crystals that melt at 205°C (Windholz et al., 1983), decompose at 222°C (Aldrich, 1992), or decompose at 195°C (NTP, 1992). Infrared and nuclear magnetic resonance spectra, as well as high-performance liquid chromatography, Karl Fischer water analysis, titration, and elemental analysis data are available for the dihydrochloride salt (NTP, 1992). The rate constant of electron attachment (k_e) for 2,4-Diaminophenol Dihydrochloride is 0.4×10^{12}/ $M^{-1}S^{-1}$ (Bakale and McCreary, 1992).

Method of Manufacture

2,4-Diaminophenol is prepared by the reduction of 2,4-dinitrophenol with either tin hydrochloride or phosphorus iodide in water. The salt is formed from the free-base (NTP, 1992).

Impurities

A chromatographic analysis of 2,4-Diaminophenol (grade not specified) resulted in the identification of one impurity, 2-amino-4-nitrophenol, which was present at a concentration of 2.7% (NTP, 1992).

USE

Cosmetic Use

The only reported cosmetic uses of 2,4-Diaminophenol and 2,4-Diaminophenol Dihydrochloride are as hair colorants (Nikitakis, 1988). Data submitted to the Food and Drug Administration (FDA) in 1993 by cosmetic firms participating in the voluntary cosmetic registration program indicated that 2,4-Diaminophenol Dihydrochloride was used in three hair dyes and colors. There were no reported uses of 2,4-Diaminophenol (FDA, 1993).

Hair-coloring formulations containing 2,4-Diaminophenol or 2,4-Diaminophenol Dihydrochloride 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 2,4-Diaminophenol or 2,4-Diaminophenol Dihydrochloride several times a day.

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).

The oxidative or permanent hair dyes containing 2,4-Diaminophenol or 2,4-Diaminophenol Dihydrochloride, as "coal tar" hair products, are exempt from the principal adulteration provision and from the color additive provision in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a caution statement and "patch test" instructions for determining whether the product causes skin irritation (Federal Register, 1979). In order to be exempt, the following caution statement must be displayed on all coal tar hair dye products:

> Caution—This product contains ingredients which may cause skin irritation on certain individuals and a preliminary test according to accompanying directions should be made. This product must not be used for dyeing the eyelashes or 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 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 of patients for sensitization (allergic contact dermatitis) should be conducted by the procedures used by the North American Contact Dermatitis Group and the International Contact Dermatitis Group (North American Contact Dermatitis Group, 1980; Eiermann et al., 1982; Adams et al., 1985). Basically, these procedures state that the test material should be applied at an acceptable concentration to the patient, covered with an appropriate occlusive patch, and evaluated for sensitization at 48 and 72 hours after application. The CIR Expert

Panel has cited the results of studies conducted by both the North American Contact Dermatitis Group and the International Contact Dermatitis Group in its safety evaluation reports on cosmetic ingredients (Elder, 1985).

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

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

International Use

2,4-Diaminophenol Dihydrochloride is approved for use in Japan in cosmetic formulations for which ingredient labeling is required (CTFA, 1987).

In the European Economic Community (EEC), Diaminophenols are approved for both general and professional use as oxidizing coloring agents for hair dyeing at concentrations not to exceed 10%, calculated as free base, with a warning statement printed on the label (EEC, 1989).

Noncosmetic Use

2,4-Diaminophenol Dihydrochloride is used as a photographic developer and as an intermediate in the process of dyeing fur (Windholz et al., 1983; NTP, 1992).

TOXICOLOGY

Intraperitoneal Toxicity

The intraperitoneal lowest reported lethal dose (LD_{L_o}) for 2,4-Diaminophenol was 50 mg/kg (Revue Belge de Pathologie et de Medecine Experimentale, 1952).

Oral Toxicity

Acute Toxicity

Groups of five male and five female Wistar albino rats received single oral doses of 0.14, 0.20, 0.29, 0.41, 0.84, and 5.0 g/kg 2,4-Diaminophenol Dihydrochloride; the control group also consisted of ten rats. Animals were observed 1, 2, and 4 h postdosing and twice a day for 2 weeks thereafter. Body weights were recorded weekly. After 2 weeks, survivors were killed for necropsy. All animals of the 0.41-g/kg and greater dose groups had died by the end of Day 2. Deaths observed in lower-dose groups prior to the end of Day 2 were as follows: 0.29 mg/kg dose (two male and four female), 0.20 mg/kg dose (one male and one female), and 0.14 mg/kg dose (one male). These represent all of the deaths that were observed at doses of $\leq$0.29 mg/kg. Each of the groups consisted of 5 males and 5 females. The six groups received the six doses indicated, respectively. Clinical signs included lethargy, ataxia, diarrhea, piloerection, ptosis, flaccid muscle tone, prostration, negative righting reflex, vocalization upon examination, and staining of the ano-

genital area. Upon necropsy, abnormalities of the lungs, liver, spleen, and gastrointestinal tract were observed. Alopecia, emaciation, chromorhinorrhea, and wetness of the anogenital area were signs observed in the survivors. Weight gain in survivors was comparable to that observed in controls. The LD_{50} for 2,4-Diaminophenol Dihydrochloride was 0.24 g/kg (Polaroid, 1989).

Short-Term Toxicity

Doses of 6, 13, 25, 50, and 100 mg/kg 2,4-Diaminophenol Dihydrochloride were mixed with corn oil and administered daily by gavage to F344/N rats, five animals of each sex per dose group. The control group consisted of five rats. $B6C3F_1$ mice (five per group) were dosed with 13, 25, 50, 100, and 200 mg/kg 2,4-Diaminophenol Dihydrochloride according to the same procedure; the control group consisted of five mice. Doses were administered on Days 1–5, 8–12, and then for 2 consecutive days, after which animals were killed. The animals were weighed on Days 1, 8, and 16 and observed twice daily. On Day 16, animals were killed for necropsy.

All of the rats survived to the end of the study. Mean body weights were not affected by the test material. Renal tubular cell necrosis (mild to moderate in severity) was observed in four male and five female rats of the 100-mg/kg dose group, three male and one female rat of the 50-mg/kg dose group, and three male and five female rats of the 25-mg/kg dose group. The lesion was considered to be related to the test substance.

In mice, survival of the highest dose groups was reduced. In the 200-mg/kg dose group, five male and four female mice died before the end of the study. In the 100-mg/kg dose group, five male and three female mice died, and three deaths (female) were observed in the 50-mg/kg dose group. Mean body weights of surviving animals were similar to those noted in controls. Relative and absolute liver weights of females dosed with 50 or 100 mg/kg were significantly increased over control values. Renal tubular cell necrosis was observed in four male and five female mice of the 100-mg/kg dose group (NTP, 1992).

Subchronic Toxicity

The NTP (1992) performed 13-week studies using F344/N rats and $B6C3F_1$ mice. Groups consisted of 10 animals of each sex. Experimental rats received doses of 12, 25, 50, 100, and 200 mg/kg 2,4-Diaminophenol Dihydrochloride in corn oil; mice received 5, 9, 19, 38, and 75 mg/kg. Negative control groups of rats and mice each consisted of 20 animals. Doses were administered 5 days a week for 13 weeks by gavage. All animals were observed twice daily; moribund animals were killed. Weights were measured and clinical examinations were performed weekly. At the end of the studies, necropsy and histopathological examination of a variety of tissues were performed.

In the rat study, 9 of 10 male and 10 of 10 female rats of the 200-mg/kg dose group died before the end of the study; in the 100-mg/kg dose group, 4 male and 1 female died. Mean body weights were reduced for male rats of the 50-mg/kg

(11%) and 100-mg/kg (21%) dose groups, and female rats of the 100-mg/kg dose group (4%). Diarrhea and lethargy were observed in rats of the two highest dose groups. The relative kidney weights were significantly increased in all experimental groups of male rats, and in female rats dosed with 50 mg/kg and 100 mg/kg 2,4-Diaminophenol Dihydrochloride. Absolute kidney and absolute and relative liver weights were also increased in female rats that received doses of 50 mg/kg and 100 mg/kg, respectively. A significant, dose-dependent increase in renal cortical tubular necrosis was observed in male rats given ≥25 mg/kg doses of 2,4-Diaminophenol Dihydrochloride and in female rats given doses of ≥100 mg/kg. The necrosis was severe in rats of the two highest dose groups, while minimal necrosis associated with regenerative tubular epithelium was reported in the lower-dose groups. A dose-related increase in granular brown pigment (which did not stain positive for iron) was observed in renal tubular cells of female rats in all experimental groups and in male rats dosed with 50, 100, and 200 mg/kg.

In the nonglandular stomach, ulcers usually associated with severe acute inflammation were observed in male rats that received 50, 100, and 200 mg/kg and in female rats that received 100 and 200 mg/kg. Epithelial hyperplasia extending into the gastric wall was associated with these ulcers. Acanthosis and hyperkeratosis of the squamous epithelium of the nonglandular stomach were also observed. A few rats in each of the higher-dose groups had foci of spongiosis in the stratum corneum or separation between the stratum corneum and the overlying keratin layer. Macrophages in the duodenal lamina propria contained a black-brown pigment (which did not stain positive for iron) in almost all treated rats. Golden brown pigment (which did stain positive for iron) and swollen Kupffer's cells, phagocytized erythrocytes, and bone marrow hyperplasia were observed in male rats given 100 and 200 mg/kg. There was a dose-dependent increase in the severity of splenic myeloid hyperplasia in treated male rats and splenic hemosiderosis in female rats. Significant splenic lymphoid depletion was observed in male rats given 100 and 200 mg/kg (NTP, 1992).

In the mouse study, all female mice survived to the end of the study. For male mice, all but one from the control group, two from the 5 mg/kg group, one from the 19 mg/kg group, and one from the 75 mg/kg group survived to the end of the study. Mean body weight gains were similar in dosed and control groups for both sexes. Absolute and relative liver and kidney weights were increased in all female treatment groups and in male mice given doses of 19, 38, and 75 mg/kg. An increase in absolute heart weight was observed in female rats from the three highest dose groups. Multifocal and diffuse renal cortical tubular regeneration were observed in mice of both sexes dosed with 75 mg/kg; the severity of these lesions was greater in female mice. In the same dose groups, renal cortical tubular cells had granular brown pigment. In the nonglandular stomach, acanthosis and hyperkeratosis were observed in male and female mice dosed with 38 and 75 mg/kg 2,4-Diaminophenol Dihydrochloride, respectively. In male and female mice of the 9-, 19-, 38-, and 75-mg/kg dose groups, macrophages in the duodenal lamina propria contained a black-brown pigment. Swollen Kupffer's cells with brown pigment were observed in female mice given doses of 38 and 75 mg/kg. Splenic hemosiderosis was increased in all dose groups (NTP, 1992).

Chronic Toxicity

Two-year bioassays with 2,4-Diaminophenol Dihydrochloride in rats and mice were completed by NTP (1992). In F344/N rats, groups of 60 animals of each sex were given doses of either 12.5 or 25 mg/kg 2,4-Diaminophenol Dihydrochloride in corn oil (by gavage) 5 days a week for up to 103 weeks. In $B6C3F_1$ mice, groups of 60 animals of each sex were given doses of either 19 or 38 mg/kg 2,4-Diaminophenol Dihydrochloride in corn oil according to the same procedure. Observations were made twice per day, and all moribund animals were killed for necropsy. The animals were weighed weekly for the initial 13 weeks and monthly for the remainder of the study. After 15 months, blood was drawn from 10 animals in each dose and control group (mice and rats of both sexes) and the animals were killed for necropsy. Microscopic examination of a variety of tissues was performed, at 15 months, for all animals of the high-dose and control groups; some tissues from the low-dose animals were examined at this time as well. At the end of the study, all surviving animals were killed for necropsy. All organs and tissues were examined for gross lesions, and all major tissues were fixed, sectioned, and stained for histopathological evaluation. Microscopic examination was also performed on tissues af all animals that died or were killed moribund between months 15–21. Selected tissues from all other animals were submitted for microscopic examination.

In the rat bioassay, there was little difference in survival among experimental groups of male and female rats when they were compared with their respective controls; however, female rats had a greater rate of survival. Mean body weights of male rats dosed with 25 mg/kg were 7–16% lower than those of controls after 25 weeks. For female rats, mean body weights in the 25-mg/kg dose group were up to 11% lower than those of controls after 37 weeks. Mean body weights of 12.5-mg/kg male rats were slightly lower than those of controls, but were generally within 5% of controls, whereas those of the 12.5-mg/kg female rats were similar to controls. No adverse signs in the hematological or clinical chemistry data were noted at 15 months in either dose group. Dose-related increases in the incidence and severity of nephropathy in female rats and in the severity of nephropathy in male rats were noted.

A golden brown, iron-positive pigment (hemosiderin) was present in the cytoplasm of the proximal renal tubule epithelial cells and, occasionally, was also present in the lumina of the proximal renal tubules of male and female rats of both dose groups. Pigment was also observed in the mucosal cells of the duodenum, stomach, and macrophages of the pancreatic and mesenteric lymph nodes of dosed rats. The pigment was determined to be either 2,4-Diaminophenol Dihydrochloride or a metabolite. In the nonglandular stomach, ulcers usually associated with severe acute inflammation were observed in all dose groups. Epithelial hyperplasia extending into the wall of the stomach was associated with these ulcers. Acanthosis and hyperkeratosis of the gastric squamous mucosa were also observed. A few rats in each of the higher dose groups had foci of spongiosis in the stratum corneum or separation between the stratum corneum and the overlying keratin layer. Pigmented macrophages, containing a combination of hemo-

siderin and either 2,4-Diaminophenol Dihydrochloride or a metabolite, were observed in the cortex and medullary cords of the lymph nodes. The incidence and description of tumors in this study are summarized in the Carcinogenicity section of this review (NTP, 1992).

In the mouse bioassay, there was little or no significant difference between control and treated mice in survival or mean body weight gain, with the exception of the 38-mg/kg male dose group, for which a slight reduction in weight gain (6%) was reported. At 15 months, dosed animals had significant reductions in hematocrit, hemoglobin, and erythrocyte counts. Animals of the highest dose groups had significant increases in leukocyte and segmented neutrophil counts. Clinical chemistry parameters for the dosed groups were comparable to those of controls. Renal tubular necrosis and regeneration were observed in all dosed animals, but not in controls. The cytoplasm of proximal renal tubule epithelial cells contained a golden brown pigment that stained positive for iron in dosed mice of both sexes. Pigment was also observed in the duodenum, liver, and mesenteric lymph nodes of dosed mice. The pigment was a combination of hemosiderin and either 2,4-Diaminophenol Dihydrochloride or a metabolite. Dosed male mice had an increased incidence of ulcers and acanthosis of the nonglandular stomach. The incidence and description of tumors in this study are summarized in the Carcinogenicity section of this review (NTP, 1992).

Dermal Irritation

2,4-Diaminophenol Dihydrochloride, 0.5 g per test site, was applied to a 6-cm^2 clipped area on the backs of three New Zealand albino rabbits. Sites were covered with semi-occlusive patches for 4 h, after which the patches were removed and the sites washed. Reactions were scored on a scale of 0–4 for erythema and edema at 1, 24, 48, and 72 h after patch removal. At 1 and 24 h, all animals had well-defined erythema and slight-to-moderate edema. At 72 h, only erythema was observed in the three animals; one animal had very slight edema. 2,4-Diaminophenol Dihydrochloride was considered to be irritating, but not corrosive, to the skin of rabbits (Polaroid, 1989).

The skin-irritation potential of 2,4-Diaminophenol Dihydrochloride was evaluated using 10 Hartley albino guinea pigs. Concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, and 10.0% in white petrolatum were applied for 48 h, under an occlusive patch, to the flanks of each animal. Skin irritation was not induced at any of the concentrations tested (Ishihara et al., 1985).

Dermal Sensitization

The skin-sensitization potential of 2,4-Diaminophenol Dihydrochloride was evaluated using 10 Hartley albino guinea pigs. During induction, 1.0% 2,4-Diaminophenol Dihydrochloride in white petrolatum (~50 mg) was applied, under an occlusive patch, to the nape for 48 h. This procedure was repeated three times per week for 2 weeks. After a 2-week nontreatment period, challenge concentrations of ~0.1% and 1.0% were applied, under an occlusive patch, to the flanks for

48 h. Reactions were scored at 24 and 48 h after patch removal. 2,4-Diaminophenol Dihydrochloride did not induce skin sensitization (Ishihara et al., 1985).

Ocular Irritation

2,4-Diaminophenol Dihydrochloride, 0.1 ml equivalent, was placed into the conjunctival sac of one eye in each of three New Zealand albino rabbits; eyes were not rinsed. The ingredient was tested as received (gray-green powder). Reactions were scored according to the Draize scale at 1, 24, 48, and 72 h postinstillation. Additionally, after 24 h, the eyes were stained with sodium fluorescein to determine the extent of any corneal damage. Throughout the study, severe conjunctival irritation, iritis, corneal opacity, and chemosis were observed in the three animals. 2,4-Diaminophenol Dihydrochloride was an ocular irritant and an ocular corrosive agent (Polaroid, 1989).

DEVELOPMENTAL TOXICITY

A permanent hair dye formulation containing 0.2% 2,4-Diaminophenol 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 the shaved area on Days 1, 4, 7, 10, 13, 16, and 19 of gestation. A positive control group received acetylsalicylic acid by gavage, and three negative control groups were shaved but received no treatment. All animals were killed on Day 20. No significant differences in the mean number of corpora lutea, live fetuses, and resorptions per pregnancy were reported for the experimental animals. There were no significant changes in soft-tissue or skeletal anomalies between the fetuses of experimental and negative control groups (Burnett et al., 1976).

GENOTOXICITY

2,4-Diaminophenol Dihydrochloride neither induced chromosomal aberrations in Chinese hamster ovary (CHO) cells at concentrations of 0.09–0.9 μg/ml without metabolic activation nor at concentrations of 0.9–9 μg/ml with metabolic activation. The test substance neither induced sister chromatid exchanges in CHO cells at concentrations of 0.27–2.7 μg/ml without metabolic activation nor at concentrations of 0.9–9 μg/ml with metabolic activation (Galloway et al., 1987).

In the absence of S9 metabolic activation, a concentration of 2 μg/ml 2,4-Diaminophenol Dihydrochloride caused a 1.8-fold increase in forward mutations in L5178Y tk^+/tk^- mouse lymphoma cells. The 4 μg/ml test concentration was too toxic to the cell line, and determination of mutagenicity was not possible at this concentration (McGregor et al., 1987).

2,4-Diaminophenol Dihydrochloride (3 μg/plate) was mutagenic with, but not without, S9 metabolic activation in strain TA98 of *Salmonella typhimurium*. Mutagenicity was slightly enhanced when the test substance was mixed with H_2O_2 (Watanabe et al., 1989).

A sex-linked recessive lethal test was performed using *Drosophila melanogaster*. 2,4-Diaminophenol Dihydrochloride was first assayed in an adult

feeding experiment. A retest, which involved injection of adult flies with the test substance, was also conducted. Results of the initial test were inconclusive for 2,4-Diaminophenol Dihydrochloride at a test concentration of 3,500 ppm in the diet. Results of the retest indicated that 2,4-Diaminophenol Dihydrochloride was not mutagenic at a concentration of 125 ppm (Zimmering et al., 1985).

CARCINOGENICITY

A permanent hair dye formulation containing 0.2% 2,4-Diaminophenol was applied topically, 0.05 ml volume, to the clipped intrascapular area of Swiss-Webster mice (50 males, 50 females) once weekly for 23 months. At 7 and 9 months, 10 male and 10 female mice from the experimental group and from each of the three untreated control groups were killed and necropsied. Gross and microscopic examinations were performed on all mice that died during the study or that were killed at the end of the experiment. The incidences of neoplasms in control and treated groups were similar. Carcinogenic effects were not induced by the hair dye formulation (Burnett et al., 1980).

The carcinogenicity of 2,4-Diaminophenol Dihydrochloride was evaluated in NTP (1992) 2-year rat and mouse bioassays (See Chronic Toxicity section of this review for additional details). In F344/N rats, groups of 60 animals (5–6 weeks old) of each sex were dosed by gavage with 12.5 or 25 mg/kg 2,4-Diaminophenol Dihydrochloride in corn oil 5 days a week for up to 105 weeks. In B6C3F_1 mice, groups of 60 animals of each sex (6 weeks old) were dosed with 19 or 38 mg/kg 2,4-Diaminophenol Dihydrochloride in corn oil over the same period. After 15 months, blood samples were obtained from 10 animals in each experimental and control group (male and female rats and mice), and the animals were then killed for necropsy. Animals were observed twice a day, and all classified as moribund were killed. The animals were weighed weekly for the initial 13 weeks, and, monthly, for the remainder of the study.

At the end of the study, all surviving animals were killed and necropsied. All gross lesions and major tissues were fixed, sectioned, and stained for histopathological evaluation. Microscopic examination of a variety of tissues was performed at 15 months on all animals of the high-dose and control groups. Some tissues from low-dose animals were examined at this time as well. Microscopic examinations were performed on all animals that died or were killed moribund between months 15–21. Selected tissues from the remaining animals were submitted for microscopic examination.

An increased incidence of focal renal tubular cell hyperplasia was reported for male and female rats of the high-dose (25-mg/kg) groups. Because of this increase, which was significant, additional serial sections of residual formalin-fixed kidneys from all high-dose groups and controls were examined. The combined microscopic observations for kidney sections originally evaluated and additional serial kidney sections were as follows (50 animals in each group were examined): For male rats, three control and ten high-dose rats had renal tubular cell focal hyperplasia, no controls and 3 high-dose rats had renal tubular cell adenomas, and one control and no high-dose rats had renal tubular cell carcinomas. For female rats,

two controls and thirteen high-dose rats had renal tubular cell focal hyperplasia, no controls and one high-dose rat had renal tubular cell adenoma, and none of the animals had renal tubular cell carcinoma (see Table 1).

Mice of both sexes in the high-dose (38 mg/kg) groups had an increased incidence of renal tubular focal hyperplasia and adenomas. Because of this increase, additional serial sections of residual formalin-fixed kidneys from all high-dose groups and controls were prepared and examined; this was not done in histopathological evaluations of mice in low-dose (19 mg/kg) groups. The combined microscopic observations for kidney sections originally evaluated and additional serial kidney sections are as follows: for male mice, none of fifty controls and nine of fifty high-dose mice had renal tubular cell focal hyperplasia and none of fifty controls and six of fifty high-dose mice had renal tubular cell adenomas. For female mice, none of fifty controls and three of sixty high-dose mice had renal tubular cell focal hyperplasia and none of fifty controls and one of the fifty high-dose mice had renal tubular cell adenoma (see Table 2). Renal tubular cell carcinoma was observed in one of 39 female mice in the low-dose group (19 mg/kg), but not in control or high-dose female mice.

NTP (1992) concluded that "under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity* of 2,4-Diaminophenol Dihydrochloride in male or female F344/N rats that received 12.5 or 25 mg/kg. There was *some evidence of carcinogenic activity* of 2,4-Diaminophenol Dihydrochloride in male $B6C3F_1$ mice based on increased incidences of renal tubular cell adenomas; there was *no evidence of carcinogenic activity* of 2,4-Diaminophenol Dihydrochloride in female $B6C3F_1$ mice that received 19 or 38 mg/kg."

EPIDEMIOLOGY

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

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

TABLE 1. *Lesions of the renal tubule in F344/N rats administered 25 mg/kg 2,4-Diaminophenol Dihydrochloride (National Toxicology Program, 1992)*

	Male		Female	
Lesion	Control	25 mg/kg	Control	25 mg/kg
Focal hyperplasia	3/50	10/50	2/50	13/50
Adenoma	0/50	3/50	0/50	1/50
Carcinoma	1/50	0/50	0/50	0/50

TABLE 2. *Lesions of the renal tubule in B6C3F$_1$ mice administered 38 mg/kg 2,4-Diaminophenol Dihydrochloride (National Toxicology Program, 1992)*

Lesion	Male		Female	
	Control	38 mg/kg	Control	38 mg/kg
Focal hyperplasia	0/50	9/50	0/50	3/50
Adenoma	0/50	6/50	0/50	1/50
Carcinoma	0/50	0/50	0/50	0/50

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

The IARC Working Group concluded that "there is *inadequate evidence* that personal use of hair colourants entails exposures that are carcinogenic." Hence, "personal use of 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

2,4-Diaminophenol and 2,4-Diaminophenol Dihydrochloride, aromatic amines, are hair colorants in oxidative hair dyes. Current (1993) FDA frequency-of-use data indicate that 2,4-Diaminophenol Dihydrochloride is used in three hair dyes and colors; there are no reported uses of 2,4-Diaminophenol. The former ingredient is prepared by the reduction of 2,4-dinitrophenol with either stannous hydrochloride or phosphorus iodide in water.

The acute oral LD_{50} for 2,4-Diaminophenol Dihydrochloride in rats was 0.24 g/kg. 2,4-Diaminophenol Dihydrochloride induced renal toxicity in acute, short-term, subchronic, and chronic oral toxicity tests. Dose-dependent increases in renal toxicity were noted, and renal toxic effects were noted at doses as low as approximately one-ninth the LD_{50} in rats.

2,4-Diaminophenol Dihydrochloride induced skin irritation in albino rabbits. This ingredient was also a severe ocular irritant when instilled into the eyes of albino rabbits.

2,4-Diaminophenol Dihydrochloride did not induce skin irritation in albino guinea pigs patch tested with concentrations ranging from 0.2 to 10.0% in petrolatum. This ingredient also did not induce sensitization in guinea pigs challenged with concentrations of ~0.1% and 1.0% in petrolatum.

The administration of a permanent dye formulation containing 0.2% 2,4-Diaminophenol to female rats up to Day 19 of gestation did not result in teratogenic effects.

2,4-Diaminophenol Dihydrochloride was not genotoxic to CHO cells at concentrations up to 0.9 μg/ml (without metabolic activation) and at concentrations up to 9 μg/ml (with metabolic activation). Negative results were also reported when this ingredient was evaluated in the sex-linked recessive lethal test, using *D. melanogaster*. However, 2,4-Diaminophenol Dihydrochloride (2 μg/ml) induced forward mutations in mouse lymphoma cells (without metabolic activation) and, at a concentration of 3 μg/plate, was mutagenic to strain TA98 of *S. typhimurium*.

A permanent dye formulation containing 0.2% 2,4-Diaminophenol was not carcinogenic when dermally applied to Swiss Webster mice once weekly for 23 months.

Based on 2-year oral (gavage) bioassays in rats and mice, NTP concluded that there was no evidence of carcinogenic activity of 2,4-Diaminophenol Dihydrochloride in male or female F344/N rats that received doses of 12.5 or 25 mg/kg. NTP also concluded that there was some evidence of carcinogenic activity of 2,4-Diaminophenol Dihydrochloride in male $B6C3F_1$ mice that received doses of 38 mg/kg. This conclusion is based on an increased incidence of renal tubular cell adenomas. There was no evidence of carcinogenic activity of 2,4-Diaminophenol Dihydrochloride in female $B6C3F_1$ mice that received doses of 19 or 38 mg/kg.

DISCUSSION

The CIR Expert Panel is aware of the sensitization potential of hair dyes, but also recognizes that the oxidative or permanent hair dyes containing 2,4-Diaminophenol Dihydrochloride, as "coal tar" hair dye products, are exempt from the principal adulteration provision and from the color additive provision in Sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a caution statement and "patch test" instructions.

The Panel also expressed concern over what was considered to be a high concentration (2.7%) of 2-amino-4-nitrophenol, an impurity in 2,4-Diaminophenol. However, because a permanent hair dye formulation containing 0.2% 2,4-Diaminophenol was not carcinogenic in a 23-month dermal carcinogenicity study, the Panel agreed that any further concern relating to the carcinogenicity of 2-amino-4-nitrophenol, as an impurity in 2,4-Diaminophenol, in cosmetics is not warranted.

CONCLUSION

On the basis of the data included in this report, the CIR Expert Panel concludes that 2,4-Diaminophenol and 2,4-Diaminophenol Dihydrochloride are safe for use in hair dyes at concentrations up to 0.2% (as the free base).

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