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Final Report on the Safety Assessment of *N*-Phenyl-*p*-Phenylenediamine, *N*-Phenyl-*p*-Phenylenediamine Hydrochloride, and *N*-Phenyl-*p*-Phenylenediamine Sulfate[1]

Abstract: *N*-Phenyl-*p*-Phenylenediamine and its sulfate and hydrochloride salts are aromatic amines. Whereas *N*-Phenyl-*p*-Phenylenediamine Sulfate is not currently reported to be used in cosmetics, *N*-Phenyl-*p*-Phenylenediamine and *N*-Phenyl-*p*-Phenylenediamine Hydrochloride are used as colorants in hair dyes/colors. Data evaluated are for *N*-Phenyl-*p*-Phenylenediamine and are extended to the sulfate and hydrochloride salts. The oral median lethal dose (LD_{50}) in rats ranged from 464 mg/kg to 1,000 mg/kg. Acute oral toxicity studies in cats showed increased methemoglobin and Heinz body formation ≥25 mg/kg. Dose-dependent weight reductions were seen in rats fed ≥2,200 ppm for 90 days. Mice fed 14,700 ppm showed a similar response. CNS damage was observed in mice fed ≥5,000 ppm for 91 weeks. Irritation and sensitization were both observed in guinea pigs, but no ocular irritation was seen in rabbits. Oral administration to female rats showed no developmental toxicity in one study, but showed maternal and fetal toxicity and an increase in skeletal malformations in another. Dermal application of a hair dye formulation containing 2.0% *N*-Phenyl-*p*-Phenylenediamine to female rats and mice showed no developmental toxicity of any sort. No evidence of carcinogenicity was found in either male or female rats fed *N*-Phenyl-*p*-Phenylenediamine for 78 weeks, or in female mice fed up to 10,000 ppm for 48 weeks. Male mice, however, showed a non–dose-dependent increased incidence of hepatocellular neoplasms. In another study, dermal application of a hair dye formulation containing 2.0% *N*-Phenyl-*p*-Phenylenediamine to male and female mice once a week for 23 months resulted in no increase in neoplasms compared to controls. Clinical data indicate that *N*-Phenyl-*p*-Phenylenediamine is a skin irritant and can be a sensitizer. Sensitization reactions were more common among dermatitis patients who work as hairdressers. Products containing these ingredients are exempt from the principal adulteration provision of the Federal Food, Drug, and Cosmetic Act when the label bears a caution statement and instructions to perform a patch test to ascertain if the user is sensitive to them. Because this ingredient is a sensitizer, users should be screened with a patch test. On the basis of the information in this report, it is concluded that *N*-Phenyl-*p*-Phenylenediamine, *N*-Phenyl-*p*-Phenylenediamine Sulfate, and *N*-Phenyl-*p*-Phenylenediamine Hydrochloride are safe for use in hair dyes at concentrations up to 1.7% (as the free base). **Key Words:** *N*-Phenyl-*p*-Phenylenediamine—*N*-Phenyl-*p*-Phenylenediamine Hydrochloride—*N*-Phenyl-*p*-

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

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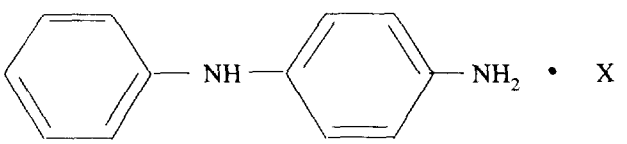
Phenylenediamine Sulfate—Safety—Cosmetic use—Hair dye—Rat—Mouse—Human—Chemistry—Toxicity—Carcinogenicity.

N-Phenyl-*p*-Phenylenediamine, *N*-Phenyl-*p*-Phenylenediamine Hydrochloride, and *N*-Phenyl-*p*-Phenylenediamine Sulfate are used as hair colorants in cosmetic formulations. The following is a summary of data available to the Cosmetic Ingredient Review (CIR) concerning the chemistry, toxicity, carcinogenicity, and cosmetic use of *N*-Phenyl-*p*-Phenylenediamine and its Sulfate and Hydrochloride salts.

CHEMISTRY

Definition and Structure

N-Phenyl-*p*-Phenylenediamine (CAS No. 101-54-2), *N*-Phenyl-*p*-Phenylenediamine Hydrochloride (CAS No. 2198-59-6), and *N*-Phenyl-*p*-Phenylenediamine Sulfate (CAS No. 4698-29-7) are aromatic compounds that conform to the following formula:



where X is either HCl or H_2SO_4, respectively. Other names for *N*-Phenyl-*p*-Phenylenediamine include 4-aminodiphenylamine; *p*-aminodiphenylamine; 1,4-benzenediamine, *N*-phenyl; CI76085; *N*-phenyl-1,4-benzenediamine; and Rodol Gray B Base. Other names for *N*-Phenyl-*p*-Phenylenediamine Hydrochloride include *p*-aminodiphenylamine HCl; 1,4-benzenediamine, *N*-phenyl, hydrochloride; CI76086; and *N*-phenyl-1,4-benzenediamine hydrochloride. Other names for *N*-Phenyl-*p*-Phenylenediamine Sulfate include *p*-aminodiphenylamine sulfate; 1,4-benzenediamine, *N*-phenyl, sulfate; *N*-phenyl-1,4-benzenediamine sulfate; and Rodol Gray BS Base (Nikitakis et al., 1991).

Physical and Chemical Properties

N-Phenyl-*p*-Phenylenediamine has a molecular weight of 184.24, a melting point of 70°C (Bayer, 1988) to 75°C (Weast, 1982), and a boiling point of 354°C (Bayer, 1988; Weast, 1982). It appears as needles in alcohol and as crystals in ligroin. It has a solubility in water of 0.6 g/L, a density of 1.09 g/cm^3, and a pH of ~7.1 in water (Bayer, 1988). It is also soluble in ether (Weast, 1982). The hydrochloride salt has a molecular weight of 220.70 (Eastman Kodak Company, 1979).

The absorption maximum for *N*-Phenyl-*p*-Phenylenediamine in Kreb's Ringer bicarbonate buffer (yellow solution, pH 7.4) was 430 nm (Raza et al., 1982).

USE

Cosmetic Use

N-Phenyl-*p*-Phenylenediamine Sulfate is not currently used in cosmetics. *N*-Phenyl-*p*-Phenylenediamine and *N*-Phenyl-*p*-Phenylenediamine HCl are used in nine and 11 cosmetic products, respectively [Federal Food and Drug Administration (FDA), 1993] (see Table 1). The only reported cosmetic uses of *N*-Phenyl-*p*-Phenylenediamine and *N*-Phenyl-*p*-Phenylenediamine Hydrochloride are in hair dyes/colors. Permanent or oxidative 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 *N*-Phenyl-*p*-Phenylenediamine, *N*-Phenyl-*p*-Phenylenediamine Hydrochloride, or *N*-Phenyl-*p*-Phenylenediamine Sulfate, as "coal tar" hair dye products, are exempt from the principal adulteration provision and from the color additive provision in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a caution statement and "patch test" instructions for determining whether the product causes skin irritation (Federal Register, 1979). In order to be exempt, the following caution statement must be displayed on all coal tar hair dye products:

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

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

Product category	Total no. of formulations in category	Total no. containing ingredient (1993)	
		N-Phenyl-*p*-Phenylenediamine	*N*-Phenyl-*p*-Phenylenediamine Hydrochloride
Hair dyes/colors (requiring a cautionary statement)		9	11
1993 Totals		9	11

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 cosmetics 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 cosmetics 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.

Noncosmetic Use

N-Phenyl-*p*-Phenylenediamine has been used in the photographic (Eastman Kodak Company, 1979) and rubber industries, as well as in pesticides, dyes, and pharmaceuticals (Tewari et al., 1989).

GENERAL BIOLOGY

Protein Binding

N-Phenyl-*p*-Phenylenediamine was incubated for 2 h (37°C and pH 7.4) with compounds containing a carboxyl group: asparagine, DL- and L-aspartic acid, cysteine, cystine, L-glutamic acid, glutamine, histamine, L-histidine, pyruvic acid, tyramine, and tyrosine. Absorbance of each sample was monitored at 450 nm. The dicarboxylic compounds, aspartic and glutamic acids, had the greatest rate of binding to *N*-Phenyl-*p*-Phenylenediamine. Binding rates for asparagine and glutamine were less (Raza et al., 1982).

N-Phenyl-*p*-Phenylenediamine (10 mg in 0.5 ml buffer, pH 8.0) was incubated with 1.0 ml of rat serum or various concentrations of bovine serum albumin (BSA) for 1 h at 37°C. Aliquots were passed over a Sephadex G-200 column. Fractions were measured for absorbance at 500 nm. Those fractions with high absorbance were pooled and concentrated. Paper and gel electrophoresis were performed on these samples. *N*-Phenyl-*p*-Phenylenediamine had a time-dependent high affinity for binding with serum globulins, and binding with BSA was also demonstrated. Dialysis against solid sucrose yielded no free *N*-Phenyl-*p*-Phenylenediamine, indicating relatively stable binding. When BSA and *N*-Phenyl-*p*-Phenylenediamine aliquots were incubated with adenine, caffeine, creatine, diphenylamine or DL-lysine, and prepared as above, no change in binding affinity was observed. DL-Aspartic acid significantly reduced the binding of BSA to *N*-Phenyl-*p*-Phenylenediamine (Raza et al., 1983).

Collagen was prepared from tail tendons of adult male albino mice (Tewari et

al., 1989). Collagen fibrils in an opaque, rigid gel were then formed. Aliquots of 2.5 mg of collagen were incubated with 0.1–1.0 μmol *N*-Phenyl-*p*-Phenylenediamine or a *N*-Phenyl-*p*-Phenylenediamine–BSA complex in a buffer (pH 8.6, 5–55°C) for 15–90 min. In some samples, *p*-chloromercuribenzoate, *N*-ethylmaleimide, mercuric chloride, or urea was added. After this, the collagen was washed with buffer three times. The effect of BSA was also studied by performing a second incubation with 0.25 or 0.50 μmol BSA for 75 min. The binding of *N*-Phenyl-*p*-Phenylenediamine to collagen occurred in both a time- and concentration-dependent manner. The optimum binding temperature was 35°C. Sulfhydryl chelating agents had no effect on binding. Increasing amounts of urea led to decreased binding of *N*-Phenyl-*p*-Phenylenediamine to collagen. The binding of *N*-Phenyl-*p*-Phenylenediamine–BSA complex to collagen was described as slight.

A buffered, saturated solution of *N*-Phenyl-*p*-Phenylenediamine (1.5 ml; pH 7.4) was incubated with a 2-ml solution of 0.25*M* amino acid (alanine, asparagine, aspartic acid, glutamic acid, lysine, proline, threonine, tyrosine, or tryptophan) for 2 h at a temperature of 40°C (Srivastava et al., 1982a). After incubation, the samples' absorbance at 450 nm was measured. Additionally, the effects of the following conditions/chemicals on the interaction between *N*-Phenyl-*p*-Phenylenediamine and aspartic acid were studied: nitrogen atmosphere, temperature (0–70°C), time (0–180 min), EDTA (100–600 μmol), and cysteine, sodium metabisulfite, sodium dithionate, and ascorbic acid (20 μmol of each of these three reducing agents). The derivative of *N*-Phenyl-*p*-Phenylenediamine and aspartic acid was separated by thin-layer chromatography (TLC). Spot tests of reactants, as well as the derivative formed, were carried out by spraying 1% ninhydrin solution on filter paper. The derivative was also subjected to the Bratton-Marshall test, which involved spraying the filter paper with hydrochloric acid, 1% sodium nitrite, and 1% *N*-(1-naphthyl)ethylenediamine dihydrochloride solution. The binding of *N*-Phenyl-*p*-Phenylenediamine to acidic amino acids, particularly glutamic acid and aspartic acid, was more efficient. For the *N*-Phenyl-*p*-Phenylenediamine–aspartic acid reaction, binding increased with temperature, up to 40°C, after which binding remained the same. At pH 4.0 and 9.2, no binding occurred. Binding increased linearly over time up to 110 min. Reducing agents and anaerobic conditions inhibited the reaction. Additionally, binding decreased with increasing concentrations of EDTA. The *N*-Phenyl-*p*-Phenylenediamine–aspartic acid derivative was stable in aqueous solution for 2–3 days, after which the two compounds began to precipitate out of solution.

In Vitro Binding

Fresh intestines, from fasted ITRC albino mice, were stripped of the mesentery manually, flushed, and stored at pH 7.4. Under nitrogen, 4 mg *N*-Phenyl-*p*-Phenylenediamine was incubated with 5 cm of intestine at pH 4.5, 7.4, or 9.0 for 30, 60, 90, 120, or 180 min with EDTA and aspartic acid. The mucosal epithelium was removed, concentrated, diluted, and measured at 400–500 nm. *N*-Phenyl-*p*-Phenylenediamine, in buffer alone (yellow in color), has an absorbance maximum of 430 nm. The incubation media turned brown and had an absorbance maximum

of 450 nm. The binding was most efficient at pH 7.4. At pH 4.5, the binding was only 35% of that noted at pH 7.5; at pH 9.0, the binding was 82%. Binding was time dependent, increasing over the period of 30–180 min. EDTA and aspartic acid slightly hindered this binding. A papain digestion was also performed on mucosal epithelium and evaluated by TLC. After TLC plates were sprayed with ninhydrin and dimethylaminocinnimaldehyde (DMAC), the *N*-Phenyl-*p*-Phenylenediamine spot was in the same place as that noted for aspartic and glutamic acids (Raza et al., 1982).

Liver slices, 0.5 mm thick, were incubated with a saturated solution of *N*-Phenyl-*p*-Phenylenediamine (0.4–2.0 mg *N*-Phenyl-*p*-Phenylenediamine/g of liver; solution pH = 7.4) at a temperature of 40°C for 1 h. The liver tissue was then homogenized and digested with papain. This digest was then applied to a silica gel G plate and developed with either 1% ninhydrin or DMAC. During the incubation period, the liver slides turned black and repeated washing did not restore the original color. The recovery of *N*-Phenyl-*p*-Phenylenediamine in the supernatant after incubation with liver slices ranged between 10–60%, and the remaining *N*-Phenyl-*p*-Phenylenediamine was associated with liver tissue (Srivastava et al., 1982*a*).

The dermal uptake and efflux kinetics of *N*-Phenyl-*p*-Phenylenediamine were evaluated with use of skin segments from male albino rats (weights = ~200 g). A 20-cm^2 area of skin was excised from each animal and sliced into segments ranging in weight from 0.1 to 0.5 g; the underlying connective tissue and subcutaneous fat were removed. After each skin segment was placed in a flask containing Kreb's ringer bicarbonate buffer, *N*-Phenyl-*p*-Phenylenediamine (in ethanol) was added at concentrations up to 2 μmol. The flasks were placed in a metabolic shaker for 60 min. At the end of the incubation period, the segments were washed and homogenized, and skin-bound *N*-Phenyl-*p*-Phenylenediamine was determined with use of the modified *p*-DMAC-organic method (Joshi et al., 1987). In efflux studies, the effect of contact time and serum dilutions (rat serum) on the mobilization of skin-bound *N*-Phenyl-*p*-Phenylenediamine into the serum was determined. The uptake of *N*-Phenyl-*p*-Phenylenediamine through the skin was dependent on the surface area of the exposed tissue, and uptake was enhanced by increasing the incubation period (optimum achieved at 75 min). Additionally, a progressive increase in uptake was observed with increasing skin weight (0.1–0.5 g). Test substance concentrations up to 2 μmol exhibited a linear uptake response, which became nonlinear between 2.5–3.5 μmol. Thereafter, the uptake response followed saturation kinetics. Increases in temperature (optimum = 40°C in 25–70°C range) and pH (optimum = pH 7.5 in favorable range of pH 7.0–8.5) also enhanced uptake. In efflux studies, undiluted serum dissociated 82.0% of the skin-bound *N*-Phenyl-*p*-Phenylenediamine. For five- and tenfold dilutions, the efficacy was 47.0 and 17.0%, respectively. The rate of mobilization of skin-bound *N*-Phenyl-*p*-Phenylenediamine into the serum gradually increased with contact time; maximum efflux occurred at 120 min. The results of this study indicate that rat skin is equipped with sites that are capable of binding *N*-Phenyl-*p*-Phenylenediamine and that the area of exposure was directly proportional to the amount of amine picked up. Furthermore, the skin-bound *N*-Phenyl-*p*-

Phenylenediamine can be effluxed from the exposure site via the systemic circulation and reach the target organ (Khanna et al., 1987).

Hepatic Metabolism

Liver slices in a buffered solution were incubated with *N*-Phenyl-*p*-Phenylenediamine, 1.25 mg compound per 1 g of liver, for 4 h at 37°C. After incubation, slices were placed in a boiling water bath for 10 min. The incubation solution was filtered off, and the amount of *N*-Phenyl-*p*-Phenylenediamine was estimated in the solution and in liver slices. A concurrent experiment was performed exactly as above, with the exception that incubation was carried out under nitrogen. In the presence of nitrogen, *N*-Phenyl-*p*-Phenylenediamine was recovered almost entirely in the solution. Aerobic conditions, however, led to the recovery of ~50% of the *N*-Phenyl-*p*-Phenylenediamine. The remaining *N*-Phenyl-*p*-Phenylenediamine was bound to the liver slices (Srivastava et al., 1982*b*).

TOXICOLOGY

Oral Toxicity

Acute Toxicity

Single doses of 100, 250, 500, 1,000, and 2,500 mg/kg *N*-Phenyl-*p*-Phenylenediamine were administered in an oily solution via gavage to groups of three to five rats. After 24 h, three of five rats in the 2,500-mg/kg dose group died; after 2 days, three of five rats in the 1,000-mg/kg dose group died. In the 250- and 500-mg/kg dose groups, no deaths occurred but weakness was observed. No signs of toxicity were observed in rats dosed with 100 mg/kg *N*-Phenyl-*p*-Phenylenediamine. The LD_{50} was ~1,000 mg/kg (Bayer, 1957).

The oral LD_{50} of *N*-Phenyl-*p*-Phenylenediamine in Wistar rats was 847 mg/kg (Singh et al., 1986).

In two other studies, the oral LD_{50}s of *N*-Phenyl-*p*-Phenylenediamine in rats were 464 mg/kg (Litton Bionetics, Inc., 1973) and 720 mg/kg (Monsanto, 1989), respectively.

The oral LD_{50} of *N*-Phenyl-*p*-Phenylenediamine in rabbits was >5,000 mg/kg (Monsanto, 1989).

Cats, one animal per dose, were given a single oral dose of 10, 25, or 100 mg/kg *N*-Phenyl-*p*-Phenylenediamine in an oily solution. After 1 h, the cat dosed with 100 mg/kg had a blood methemoglobin concentration of 47% and significant cyanosis. After 2 h, the methemoglobin concentration was 34%. The cat died 3 h after dosing. The cat dosed with 25 mg/kg had a blood methemoglobin concentration of 19% and temporary cyanosis; after 2 h, the methemoglobin concentration was 20%. No signs of toxicity were observed in the cat dosed with 10 mg/kg *N*-Phenyl-*p*-Phenylenediamine (Bayer, 1957).

N-Phenyl-*p*-Phenylenediamine, 25 mg/kg in polyethylene glycol 400, was administered orally to two cats. Before dosing and 3, 7, and 24 h after dosing, methemoglobin concentrations and Heinz bodies in the blood were measured. For

one cat, methemoglobin concentrations were 2% (before dosing), 11% (3 h after dosing), and 6% (7 h after dosing). For the other cat, methemoglobin concentrations were 0% before dosing, 7% (3 h after dosing), and 7% (7 h after dosing). The extent of Heinz body formation in the two cats was 5 and 32 per 1,000, respectively, before dosing and 27 and 86 per 1,000, respectively, after dosing (Bayer, 1985).

Short-Term Toxicity

Eight-week feeding studies on *N*-Phenyl-*p*-Phenylenediamine were conducted with use of B6C3F$_1$ mice and F344 rats [National Cancer Institute (NCI), 1978]. Groups consisted of five animals of each sex. The rats received feed containing 2,200, 3,200, 4,600, 6,800, and 10,000 ppm *N*-Phenyl-*p*-Phenylenediamine and water ad libitum for 7 weeks. Rats in the untreated control group received feed only. The mice received feed containing 3,000, 4,400, 6,500, 9,500, 14,700, 21,600, and 31,500 ppm *N*-Phenyl-*p*-Phenylenediamine and water ad libitum for 7 weeks. Negative control mice received feed only. During the final week, animals were given basal feed. All animals were observed twice daily; moribund animals were killed. Weights were measured and clinical examinations were performed weekly. Body weight gain reductions were seen in male and female rats fed diets containing 2,200 ppm (72 and 50% of controls, respectively). A dose-dependent decrease in body weight gain was observed at larger doses. Reductions in body weight gain were observed in female mice fed diets containing ≥14,700 ppm. *N*-Phenyl-*p*-Phenylenediamine did not affect body weight gain in male mice.

In another short-term study, the oral toxicity of *N*-Phenyl-*p*-Phenylenediamine was evaluated with use of five groups of 12 male rats [weight (mean ± SD) = 200 ± 20 g]. The groups were fed a powdered diet containing 0, 0.1, 0.25, 0.5, or 0.75% of the ingredient, respectively, for 90 days. Beginning at Week 10 of the study, a slight reduction in feed intake was observed. Compared with that in controls, body weight gain in all treatment groups became progressively lower; however, this effect was more pronounced at higher doses. In the 0.75% dose group, body weights remained virtually static. The overall weight gain in the 0.5% dose group was 11.7%, compared with 36.0% in the control group. A 35% increase in liver weight was also observed in the 0.5 and 0.75% dose groups. A significant decrease in red blood cell count, increased erythrocyte sedimentation rate, and lowered packed cell volume, suggesting normocytic normochromic anemia, were observed at doses of ≥0.25%. At doses of 0.5 and 0.75%, there was a significant increase in serum acid/alkaline phosphatase and glutamic-oxaloacetic transaminase/glutamic-pyruvic transaminase activities, with simultaneous depletion of these activities in the liver. These changes in enzymatic activity are suggestive of biochemical lesions of the liver. At histopathological examination, degenerative changes in the hepatic cells, together with prominent plasma cell reaction in the portal triad areas, were observed only in the 0.5 and 0.75% dose groups. Partial arrest of spermatogenesis, indicated by low activities of lactic dehydrogenase (LDH) and hyaluronidase in the 0.5 and 0.75% dose groups, was also noted. At histopathological examination, patchy degeneration of seminiferous tubules (affecting sper-

matogonia, spermatocytes, spermatids, and sperm) was observed only in the 0.75% dose group (Singh et al., 1986). (See section on Reproductive Toxicity for similar reproductive effects.)

Chronic Toxicity

Ninety-one-week bioassays (oral feeding) on *N*-Phenyl-*p*-Phenylenediamine were conducted with use of F344 rats and B6C3F$_1$ mice (NCI, 1978). The compound is generally referred to as *N*-Phenyl-*p*-Phenylenediamine in these studies, with the exception that it is referred to as *N*-Phenyl-*p*-Phenylenediamine Hydrochloride in the section on chemical structure. In male and female F344 rats, groups of 50 animals per sex were fed diets containing 600 and 1,200 ppm *N*-Phenyl-*p*-Phenylenediamine for 78 weeks. Groups of 50 female B6C3F$_1$ mice were fed diets of 5,000 and 10,000 ppm *N*-Phenyl-*p*-Phenylenediamine for 31 weeks, and groups of 50 male B6C3F$_1$ mice were fed diets of 2,500 and 5,000 ppm *N*-Phenyl-*p*-Phenylenediamine for 31 weeks. Doses were reduced to 1,250 and 2,500 ppm in both sexes of mice for an additional 17 weeks. Time-weighted average doses were 3,672 and 8,170 ppm for female mice and 2,057 and 4,114 ppm for male mice. Groups of 20 animals of each sex and species were retained as controls. Animals were observed twice daily and weighted and palpated at regular intervals. At the end of the studies, necropsy and microscopic examination of a variety of tissues were performed.

No significant difference in survival or mean body weight gains was observed in dosed rats, as compared with controls. In male mice, mean body weight gains were significantly lower than controls, but there was no significant increase in mortality. A significant increase in hepatic focal inflammation was observed in male mice. In female mice, mean body weight gains were significantly lower than that in controls, and a significant increase in mortality was observed. Signs indicative of central nervous system (CNS) damage were observed in ~25% of the low dose and 40% of the high-dose female mice that died.

Dermal Irritation

N-Phenyl-*p*-Phenylenediamine, 0.5 g made into a paste with water, was applied to the shaved flanks of three male albino rabbits and covered with semi-occlusive patches for 4 h. After treatment, patches were removed and test sites rinsed with water. Animals were observed at 1 h and 1, 2, 3, and 7 days postapplication. No signs of irritation were observed throughout the study (Bayer, 1982*a*).

N-Phenyl-*p*-Phenylenediamine was applied, under occlusive patches, to the shaved flanks of nine Hartley albino guinea pigs. Concentrations of 0.1, 0.2, 0.5, 1, 2, 5, and 10% in petrolatum were applied for 48 h. The 10% solution was irritating to eight of nine guinea pigs, and the 5% solution was irritating to four of nine guinea pigs. Skin irritation was not observed at lower concentrations (Ishihara et al., 1985).

Dermal Sensitization

Guinea pigs tested in the study by Ishihara et al. (1985), mentioned previously, were given an induction application of 1% *N*-Phenyl-*p*-Phenylenediamine in petrolatum. The test substance was applied, under an occlusive patch, to the shaved nape for 48 h; patches were replaced three times per week for 2 weeks. After a 2-week nontreatment period, a challenge concentration of 0.01, 0.05, 0.1, 0.2, 0.5, or 1% was applied under an occlusive patch for 48 h. Readings at 24 and 48 h were recorded. *N*-Phenyl-*p*-Phenylenediamine strongly sensitized all of the guinea pigs at concentrations of 0.1 and 1.0%.

Groups of eight male Pirbright-White guinea pigs were treated daily (5 treatment days, followed by 2 nontreatment days—procedure repeated) with doses of 1, 2, 5, and 10% *N*-Phenyl-*p*-Phenylenediamine in petrolatum. After 16 days, a challenge dose, equivalent to half of the induction dose, was applied to an untreated site and reactions were evaluated at 1, 2, 3, and 4 days. Half of the animals were sensitized. Further details concerning the conduct of this study were not included (Schäfer et al., 1978).

Ocular Irritation

N-Phenyl-*p*-Phenylenediamine, 100 mg of the powder, was instilled into the conjunctival sac of one eye of each of three female albino rabbits (Bayer, 1982*b*). Eyes were examined at 1, 24, 48, and 72 h. At 24 h, the treated cornea, stained with 1% fluorescein, was examined. Over the last three time periods, the average primary Draize irritation score was 8.1 (scale = 0 to 110). At 72 h post-instillation, no effects were observed. *N*-Phenyl-*p*-Phenylenediamine was classified as an ocular irritant.

DEVELOPMENTAL TOXICITY

Oral

On days 6–15 of gestation, mated female Sprague-Dawley rats were given daily doses of 50, 100, or 200 mg/kg *N*-Phenyl-*p*-Phenylenediamine in propylene glycol by gavage. Positive control animals each received one dose of 100,000 IU Vitamin A on Day 9. Female rats were observed daily for overt toxicity and weighed on Days 0, 6, 16, and 20. On Day 20, dams were killed by suffocation with carbon monoxide. The uterine horns were excised and the viability of the fetuses, as well as the number of resorption sites and metrial glands, were determined. Half of the fetuses were fixed in Bouin's solution for visceral examination, and the remaining fetuses were fixed in 95% isopropyl alcohol and stained with Alizarin Red S for skeletal examination. Vitamin A was teratogenic; there was at least one abnormal fetus per litter. All of the female rats completed the study. The 200-mg/kg dose group had a significant decrease in mean body weight gain. In the following nontreatment period, this group had a significant increase in mean body weight gain. No significant differences in mean fetal weights, sex ratios, or external,

visceral, or skeletal fetal abnormalities were observed when treated and control groups were compared (Picciano et al., 1984).

In another study, mated, female Charles River CD rats were given daily doses of 10, 50, or 100 mg/kg *N*-Phenyl-*p*-Phenylenediamine in corn oil, by gavage, on days 6–15 of gestation. Females were observed twice daily for signs of overt toxicity and weighed on Days 0, 6, 11, 13, 15, and 20. Examinations of the dams were performed on Days 0, 6–15 (every day), and 20. Feed consumption was recorded weekly. On Day 20, dams were killed by exsanguination under anesthesia and necropsied. The reproductive organs were excised and weighed. The number, viability, and sex of the fetuses, as well as the number of early and late resorption sites and implantation sites, were determined. If no implants were visualized, the uterus was stained with ammonium sulfide for visualization of foci. Half of the fetuses were fixed in Bouin's solution for visceral examination, and the remaining fetuses were fixed in 70% ethanol and stained with Alizarin Red S for skeletal examination.

All of the female rats completed the study. The rats of the 100-mg/kg dose group had a significant decrease in mean body weight gain and feed consumption during dosing. In the following nontreatment period, this group had a similar mean body weight gain, as compared with the control. Mean gravid uterine weights of the 100-mg/kg dose group were significantly less than those of controls. Excessive salivation was observed in dosed females. In the high-dose group, some staining of the fur in the anogenital area, as well as soft stool, were observed. No significant differences among treated and control groups were observed at necropsy. No significant differences in implantation rates, sex ratios, or external or visceral abnormalities were observed when treated and control groups were compared. Compared with controls, mean fetal weights in the 100-mg/kg dose group were significantly less. The incidence of skeletal malformations, both per fetus and per litter, was significantly increased when compared with that in controls. The skeletal malformations observed most frequently included wavy ribs, fused ribs, vertebral defects, and ossification variations. *N*-Phenyl-*p*-Phenylenediamine was considered maternally toxic and fetotoxic at a dose of 100 mg/kg, but not at 10 or 50 mg/kg. *N*-Phenyl-*p*-Phenylenediamine was not considered teratogenic (Schroeder and Daly, 1989).

Dermal

A permanent hair dye formulation containing 2.0% *N*-Phenyl-*p*-Phenylenediamine Hydrochloride was 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 on Days 1, 4, 7, 10, 13, 16, and 19 of gestation. A positive control group received acetylsalicylic acid by gavage, and three control groups were shaved but received no treatment. All animals were killed on Day 20. No significant differences in the mean number of corpora lutea, live fetuses, and resorptions per pregnancy were reported for the experimental animals. There were no significant changes in soft-tissue anomalies or skeletal variations between the fetuses of the treatment group and the negative controls. An increase in embryotoxicity (e.g., increase in teratogenicity, increase in embryo death, and

decrease in fetal weight) was observed in the positive control group. The results in the positive control group were consistent with the published literature relative to the effects of aspirin on rat fetal development (Burnett et al., 1976).

A 2-generation dermal carcinogenicity/reproduction toxicity study of a hair dye formulation containing 2.0% *N*-Phenyl-*p*-Phenylenediamine Hydrochloride was performed (Burnett and Goldenthal, 1988). The F_0 generation (see section on Carcinogenicity) was allowed to mate again, as before, and produced generation F_{1b}. Pups were counted and weighed on Days 0, 4, 14, and 21 of lactation. On Day 21, the pups were sexed and placed into groups, 20 per sex per group. These animals received dermal applications of the dye for 100 days. Animals were then mated to produce generations F_{2a} and F_{2b}; pups were counted and weighed. After the litters were weaned, five F_1 male and five F_1 female rats were killed by decapitation and necropsied. Microscopic examination of the following tissues was performed: adrenal glands, colon, heart, ileum, jejunum, kidneys, liver, lungs, ovaries, spleen, stomach, testes, thyroid gland, urinary bladder, uterus, and skin (treated area only). A third generation was attempted, but a viral infection affected reproduction (data not reported). No significant differences in fertility, gestation, survival, live births, or body weight gains were observed between test and control groups. No treatment-related changes were observed in the five male and five female F_1 animals necropsied.

Intraperitoneal Toxicity

One group of 30 adult male albino Wistar rats [weight (mean ± SD) = 175 ± 25 g] was dosed intraperitoneally with 42.5 mg/kg *N*-Phenyl-*p*-Phenylenediamine (in refined groundnut oil) daily for 180 days. Thirty control rats were dosed with refined groundnut oil according to the same procedure. Normal growth was observed in experimental and control groups throughout the study. Both LDH and hyaluronidase activities in the testes and testicular lactic acid were significantly decreased in experimental animals, indicating arrest of spermatogenesis. Histopathological examination of the testis presented patchy degeneration of seminiferous tubules. Desquamation and sloughing off of the gametogenic epithelial elements were observed in seminiferous tubules that were severely affected (Singh et al., 1992).

GENOTOXICITY

N-Phenyl-*p*-Phenylenediamine, 1,000 μg/plate with and without metabolic activation, was not mutagenic in *Salmonella typhimurium* strains TA98, TA1535, and TA1537 (McCann et al., 1975).

Concentrations of 10–3,333 μg/plate *N*-Phenyl-*p*-Phenylenediamine, with and without metabolic activation, were not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537. Concentrations >1,000 μg/plate were bactericidal (Mortelmans et al., 1986).

Concentrations of 15, 50, and 150 μg/plate *N*-Phenyl-*p*-Phenylenediamine, with and without metabolic activation, were tested in *S. typhimurium* strain TA98. These concentrations, with metabolic activation, were considered mutagenic (Yoshikawa et al., 1976).

N-Phenyl-*p*-Phenylenediamine, with metabolic activation, was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538. It was, however, positive in a Prival test (no details available) (Freeman et al., 1987).

N-Phenyl-*p*-Phenylenediamine induced neither point mutations nor chromosomal aberrations in Chinese hamster ovary cells. *N*-Phenyl-*p*-Phenylenediamine was also negative in an in vivo/in vitro rat hepatocyte DNA-repair assay. It did, however, induce S-phase DNA replication in the in vivo/in vitro rat hepatocyte DNA-repair assay (no details available) (Monsanto, 1989).

N-Phenyl-*p*-Phenylenediamine was mutagenic in L5178Y mouse lymphoma cells (no details available) (NTP, 1988).

CARCINOGENICITY

Oral Carcinogenicity

The carcinogenicity of *N*-Phenyl-*p*-Phenylenediamine was studied in NCI (1978) 91-week bioassays (oral feeding) in rats and mice (see also Chronic Toxicity section). Throughout the studies, the compound is generally referred to as *N*-Phenyl-*p*-Phenylenediamine, with the exception that it is referred to as *N*-Phenyl-*p*-Phenylenediamine Hydrochloride in the section on chemical structure. Groups of 50 male and 50 female F344 rats were fed diets containing 600 and 1,200 ppm *N*-Phenyl-*p*-Phenylenediamine for 78 weeks. Groups of 50 female $B6C3F_1$ mice were fed diets containing 5,000 and 10,000 ppm *N*-Phenyl-*p*-Phenylenediamine for 31 weeks. Groups of 50 male $B6C3F_1$ mice were fed diets containing 2,500 and 5,000 ppm *N*-Phenyl-*p*-Phenylenediamine for 31 weeks. Concentrations were reduced to 1,250 and 2,500 ppm in both sexes of mice for an additional 17 weeks. Time-weighted average doses were 3,672 and 8,170 ppm for female mice and 2,057 and 4,114 ppm for male mice. Groups of 20 animals of each sex and species were retained as controls. Animals were observed twice daily, weighed, and palpated at regular intervals. At the end of the study, necropsy and microscopic examination on a variety of tissues were performed. There was no evidence of carcinogenicity due to *N*-Phenyl-*p*-Phenylenediamine administration in either sex of Fisher 344 rats. In male mice, a greater incidence of hepatocellular neoplasms was observed, but this was not dose dependent and did not occur in female mice (Table 2). Because of the lack of a dose response, the authors did not consider that there was conclusive evidence of carcinogenicity due to *N*-Phenyl-*p*-Phenylenediamine administration in either sex of mice.

TABLE 2. *Hepatocellular adenomas or carcinomas in mice dosed with* N-*Phenyl*-p-*Phenylenediamine (National Cancer Institute, 1978)*

Parameter	Male mice			Female mice		
	Control	2,057 ppm	4,114 ppm	Control	3,672 ppm	8,170 ppm
No. of animals	20	49	50	20	49	48
Hepatocellular carcinomas	2	6	5	0	0	0
Hepatocellular adenomas or carcinomas	2	18	10	1	2	1

Dermal Carcinogenicity

A permanent hair dye formulation containing 2.0% *N*-Phenyl-*p*-Phenylenediamine Hydrochloride was applied topically (volume = 0.05 ml) to the clipped intrascapular area of each of 50 male and 50 female Swiss Webster mice (one group) once weekly for 23 months (see also Reproductive Toxicity section). At 7 and 9 months, 10 male and 10 female mice from each group were killed and necropsied. Necropsy and microscopic examination of tissues were performed on all mice that died during the study or were killed after termination of the experiment. The incidences of neoplasms in control (three groups) and experimental groups were similar. Carcinogenic effects were not induced by the hair dye formulation (Burnett et al., 1980).

A 2-generation dermal carcinogenesis/reproduction toxicity study on various dyes, including *N*-Phenyl-*p*-Phenylenediamine Hydrochloride, in formulation was performed (Burnett and Goldenthal, 1988). Sprague-Dawley rats were grouped, 40 per sex, in order to achieve similar average weights between groups. These were identified as the F_0 generation. The fur on the backs of these animals was clipped (1-in diameter) and 0.5 ml of test material applied twice per week for 100 days. The test material consisted of 1 part dye formulation [2.0% *N*-Phenyl-*p*-Phenylenediamine, 1.0% resorcinol, 1.0% *N*-methyl-*p*-aminophenol sulfate, 1.5% *m*-phenylenediamine, 1.0% *o*-phenylenediamine, 5.0% oleic acid, 3.0% isopropanol, 0.2% sodium sulfite, and 6.0% ammonia (29% in an aqueous solution)] and 1 part 6% hydrogen peroxide, mixed immediately before application. Animals were housed together for 15 days, or until evidence of conception was present. Dams were allowed to deliver naturally. Pups were counted and weighed on Days 0, 4, 14, and 21 of lactation. On Day 21, the pups were sexed and placed into groups, 60 per sex per group assigned randomly. These were identified as F_{1a}. These animals received dermal applications of the test material, as before, for 2 years. Animals were examined daily for toxicity. For the first 14 weeks, animals were weighed every week. Thereafter, weights were recorded monthly. Feed consumption was recorded on a weekly basis.

At months 3, 12, 18, and 24, blood and urine were collected from five male and five female rats. Blood was analyzed for total erythrocytes, total and differential leucocytes, total platelets, reticulocytes, hemoglobin, hematocrit, blood glucose, blood urea nitrogen, serum alkaline phosphatase, serum glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and serum creatine. The volume of urine was measured and analyzed for pH, specific gravity, albumin, glucose, bilirubin, occult blood, and sediment. After 1 year, five male and five female rats were killed by decapitation and necropsied. At the end of the study, all surviving animals were killed by decapitation and necropsied. All tumor masses and the following tissues were examined microscopically: skin, thyroid gland, lungs, gastrointestinal tract, spleen, pancreas, liver, pituitary gland, kidneys, urinary bladder, ureters, bone marrow, lymph nodes, adrenal glands, gonads, brain, skeletal muscle, and globes. No significant differences in body weight gain, survival, hematological or urine parameters, or incidences of neoplasms were observed between test and control groups.

CLINICAL ASSESSMENT OF SAFETY

Skin Irritation and Sensitization

Predictive Test

N-Phenyl-*p*-Phenylenediamine, moistened with water, was applied via a patch to the forearms of each of five volunteers. After 2 h, no irritation was observed. After 4 h, slight irritation was observed in half of the volunteers. After 24 h, all of the volunteers had marked reddening of the affected area, which returned to normal in 1 week (further details not available) (Bayer, 1957).

Provocative Tests

The skin sensitization potential of *N*-Phenyl-*p*-Phenylenediamine was evaluated in 2,669 consecutive dermatitis patients. Concentrations of 0.25, 0.5, and 2.0% in petrolatum were tested. In standard patch tests, patches were applied on the front and sides of the thigh with use of "Lyaplast Special [R]." Reactions were scored at 48 and 96 h postapplication, and then at longer intervals. Only unquestionable reactions (erythema and papules, 2+ reactions or more) were included as positive. Reactions that were observed 7 days or later postapplication were classified as delayed reactions. The incidence of positive reactions was as follows: 0.25% *N*-Phenyl-*p*-Phenylenediamine (25 of 1,282 patients tested; two had delayed reactions), 0.5% concentration (35 of 833 patients; seven had delayed reactions), and 2.0% concentration (30 of 554 patients; 17 had delayed reactions). The authors noted that the risk of sensitization appeared to have been considerably less as the test concentration of *N*-Phenyl-*p*-Phenylenediamine was reduced (Schonning, 1969).

The frequency and source of contact sensitization in 302 hairdressers with dermatitis was evaluated in a multicenter study (nine Italian centers involved) that was conducted by members of the Gruppo Italiano Ricerca Dermatiti da Contatto e Ambientali (GIRDCA). The duration of the study was from January 1985 to June 1990, and involved 43 male and 259 female patients ranging in age from 14 to 66 years (mean age = 24.6 years); 281 patients had hand dermatitis. The mean duration of employment in the hairdressing profession for the 302 study participants was 5.3 years. Patch tests were performed according to International Contact Dermatitis Research Group (ICDRG) recommendations, using Finn Chambers on Scanpor tape. Reactions were read at 2 and 3 days. Positive reactions to 0.25% *N*-Phenyl-*p*-Phenylenediamine occurred in 32 of the 302 patients, indicating a sensitization rate of 10.6%. The results of a questionnaire that was completed by 185 of the 302 patients indicated that an average of 16 hair-dyeing procedures was performed each week. Use of gloves was not particularly frequent: of 176 hairdressers surveyed, 128 used gloves when dyeing hair, but only 86 of them used gloves when washing dyed hair (Guerra et al., 1992*a*).

The frequency of sensitization reactions was evaluated in a population of hairdressers' clients patch tested with "hairdressing allergens." All of the clients had contact dermatitis, and previous treatments with hair dyes or permanent wave solutions were suspected of causing the dermatitis. The duration of this study was

from 1985 to June of 1990, and involved 261 patients (5 male, 256 female) with an average age of 43.3 years. All patients were patch tested with the GIRDCA standard series and a hairdressers' screening series; reactions were scored at 2 and 3 days. Further details concerning the patch test procedure were not provided. Positive reactions to 0.25% *N*-Phenyl-*p*-Phenylenediamine (in petrolatum) occurred in 11 of the 261 patients, indicating a sensitization rate of 4.2% (Guerra et al., 1992*b*).

Broeckx et al. (1987) studied cosmetic intolerance in 5,202 patients. Almost all patients completed either a Belgian Tri-Contact Patch-Test series or a similar patch test series. Of the original population, 107 (2.1%) had allergic reactions to a product containing *N*-Phenyl-*p*-Phenylenediamine. Of the 156 patients with allergies to cosmetics, only five had positive reactions to that product.

Sensitization reactions were induced in 10 patients after 10–15 days of exposure to *N*-Phenyl-*p*-Phenylenediamine. Biopsies of the reaction were taken, and samples were evaluated for lesions or sectioned and treated with monoclonal antibodies to characterize cell lines. At microscopic examination, the epidermis was infiltrated with mononuclear cells and minor spongiosis was also observed. "Bizarre-shaped" nuclei were observed in some of the cells. In immunohistochemistry preparations, peripheral T lymphocytes [primarily CD3 (formerly known as Leu3a) and CD8 (formerly known as Leu2a)] composed 80–87% of the cells examined; Langerhans' cells composed 8–12%, macrophages, 2–5%, and B lymphocytes, 1–5% (Tosti et al., 1986).

Over a period of 4 years, 3,300 patients suspected of having allergic contact eczema were patch tested with *N*-Phenyl-*p*-Phenylenediamine. Of these, 343 had positive reactions. Of the 73 volunteers in the hair care profession, seven had reactions to *N*-Phenyl-*p*-Phenylenediamine. Of 123 volunteers from the care and welfare profession, 19 had reactions to *N*-Phenyl-*p*-Phenylenediamine (Landthaler et al., 1981).

Between 1968 and 1983, none of the 52 contact dermatitis patients with reactions to hair care products had reactions to *N*-Phenyl-*p*-Phenylenediamine (Angelini et al., 1985).

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 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).

The charge to the IARC Working Group was to ascertain that all appropriate data had been collected and were being reviewed; to evaluate the results of the

epidemiological and experimental studies and prepare accurate summaries of the data; and to make an overall evaluation of the carcinogenicity of the exposure to humans.

The IARC Working Group concluded that "there is *inadequate evidence* that personal use of hair colourants entails exposures that are carcinogenic." Hence, "personal use of hair colourants *cannot be evaluated as to its carcinogenicity (Group 3)*. The IARC Working Group also concluded that "there is *limited evidence* that occupation as a hairdresser or barber entails exposures that are carcinogenic." Hence, "occupation as a hairdresser or barber entails exposures that *are probably carcinogenic (Group 2A)*" (IARC, 1993). The Expert Panel concludes that the relevance of the occupational data and conclusion to individuals using hair dyes is unclear.

SUMMARY

N-Phenyl-*p*-Phenylenediamine, *N*-Phenyl-*p*-Phenylenediamine Hydrochloride, and *N*-Phenyl-*p*-Phenylenediamine Sulfate are aromatic compounds that function as hair colorants in cosmetic formulations. Current (1993) FDA frequency-of-use data indicate that *N*-Phenyl-*p*-Phenylenediamine and *N*-Phenyl-*p*-Phenylenediamine Hydrochloride are used in nine and 11 hair dyes, respectively. There are no reported uses of *N*-Phenyl-*p*-Phenylenediamine Sulfate.

Acute oral LD_{50}s for *N*-Phenyl-*p*-Phenylenediamine in rats ranged from 464 mg/kg to ~1,000 mg/kg.

In an oral toxicity study involving three cats, an increase in methemoglobin concentration was observed in the two cats dosed with 25 mg/kg and 100 mg/kg *N*-Phenyl-*p*-Phenylenediamine in an oily solution, respectively, but not in the cat dosed with 10 mg/kg. In another acute study, increased methemoglobin concentration and Heinz body formation were observed in the two cats dosed with 25 mg/kg *N*-Phenyl-*p*-Phenylenediamine in polyethylene glycol 400.

In a short-term feeding study (90 days), reductions in body weight were observed in male and female rats fed diets containing 2,200 ppm *N*-Phenyl-*p*-Phenylenediamine for 8 weeks. At greater concentrations, up to 10,000 ppm, weight reductions were dose dependent. In mice, weight reductions were observed at dietary concentrations of ≥14,700 ppm. In a 90-day feeding study using male rats, partial arrest of spermatogenesis was noted at dietary concentrations of 0.5 and 0.75%. Patchy degeneration of seminiferous tubules was observed at histopathological evaluation.

In a feeding study of longer duration (91 weeks), signs indicative of CNS damage were observed in ~25.0% of the 50 mice that were fed 5,000 ppm *N*-Phenyl-*p*-Phenylenediamine and in ~40.0% of the mice fed 10,000 ppm *N*-Phenyl-*p*-Phenylenediamine.

Skin irritation was not observed when a paste consisting of 0.5 g *N*-Phenyl-*p*-Phenylenediamine and water was applied to three albino rabbits for 4 h, using semiocclusive patches. Following the dermal application (48 h; occlusive patches) of *N*-Phenyl-*p*-Phenylenediamine to nine albino guinea pigs, skin irritation was observed only at concentrations of 5 and 10% (highest concentration tested).

When the guinea pigs were challenged with *N*-Phenyl-*p*-Phenylenediamine concentrations up to 1.0%, after a 48-h induction application, strong sensitization reactions to 0.1 and 1.0% concentrations resulted.

N-Phenyl-*p*-Phenylenediamine did not induce ocular irritation when instilled into the eyes of female albino rabbits.

Patchy degeneration of seminiferous tubules was observed in male rats dosed intraperitoneally with 42.5 mg/kg *N*-Phenyl-*p*-Phenylenediamine daily for 180 days.

Compared with the control group, daily doses of 50, 100, or 200 mg/kg *N*-Phenyl-*p*-Phenylenediamine in propylene glycol, orally administered to female rats on gestation Days 6–15, did not induce significant differences in mean fetal weights and sex ratios or in external, visceral, and skeletal fetal abnormalities. In a similar study in which female rats were dosed with *N*-Phenyl-*p*-Phenylenediamine in corn oil (doses up to 100 mg/kg), maternal and fetal toxicity were noted at the highest dose tested. Additionally, the incidence of skeletal malformations was significantly increased over that observed in the control group.

In another teratogenicity study in which a permanent dye formulation containing 2.0% *N*-Phenyl-*p*-Phenylenediamine was applied to the skin of rats on Days 1 through 19 of gestation, there were no significant changes in soft-tissue anomalies and skeletal variations between negative control and experimental groups. When the same hair dye formulation was applied to the skin of mice in a 2-generation reproductive toxicity study, there were no significant differences in fertility, gestation, survival, live births, or body weight gain between experimental and control groups.

There was no evidence of carcinogenicity in groups of male and female rats that were fed *N*-Phenyl-*p*-Phenylenediamine (600 and 1,200 ppm) in the diet for 78 weeks. Evidence of *N*-Phenyl-*p*-Phenylenediamine–induced carcinogenicity in male and female mice was inconclusive. Female mice were fed concentrations up to 10,000 ppm, and male mice, concentrations up to 5,000 ppm in the diet over a period of 48 weeks. A greater incidence of hepatocellular neoplasms was observed in male mice; however, this observation was not dose-dependent.

In another study, dermal application of a permanent hair dye formulation containing 2.0% *N*-Phenyl-*p*-Phenylenediamine to male and female mice once weekly for 23 months resulted in a similar incidence of neoplasms between experimental and negative control groups.

At 24-h postapplication, skin irritation reactions were observed in all of the five volunteers patch tested with aqueous *N*-Phenyl-*p*-Phenylenediamine. All reactions had cleared by the end of the next week.

Concentration-dependent skin irritation reactions were observed in subgroups within a population of 2,669 dermatitis patients patch tested with concentrations of *N*-Phenyl-*p*-Phenylenediamine up to 2.0%. At the highest concentration (2.0%), sensitization reactions were observed in 30 of 554 patients. In the largest patient study included in this report, 107 of 5,202 patients had allergic reactions to a cosmetic product containing *N*-Phenyl-*p*-Phenylenediamine.

Sensitization reactions were observed in 32 of 302 dermatitis patients (hairdressers) patch tested with 0.25% *N*-Phenyl-*p*-Phenylenediamine. Sensitization

reactions were also observed in 11 of 261 dermatitis patients patch tested with 0.25% *N*-Phenyl-*p*-Phenylenediamine. These patients constituted a population of hairdressers' clients.

DISCUSSION

Toxicological data on *N*-Phenyl-*p*-Phenylenediamine Hydrochloride and *N*-Phenyl-*p*-Phenylenediamine Sulfate are limited. However, the CIR Expert Panel believes that the available data on *N*-Phenyl-*p*-Phenylenediamine are sufficient for evaluating the safety of these two ingredients. *N*-Phenyl-*p*-Phenylenediamine Hydrochloride and *N*-Phenyl-*p*-Phenylenediamine Sulfate are salts of *N*-Phenyl-*p*-Phenylenediamine, and all three ingredients have essentially the same degree of biological activity.

The Expert Panel recognizes that *N*-Phenyl-*p*-Phenylenediamine is a sensitizer and that some persons may be sensitized under intended conditions of use. However, it is understood that the oxidative or permanent hair dyes containing *N*-Phenyl-*p*-Phenylenediamine, 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. Because this ingredient is a sensitizer, users should be screened with a patch test. The Panel also noted that hair dye formulations containing 2.0% *N*-Phenyl-*p*-Phenylenediamine Hydrochloride were not carcinogenic in dermal carcinogenicity studies.

CONCLUSION

On the basis of the data included in this report, the CIR Expert Panel concludes that *N*-Phenyl-*p*-Phenylenediamine, *N*-Phenyl-*p*-Phenylenediamine Hydrochloride, and *N*-Phenyl-*p*-Phenylenediamine Sulfate are safe for use in hair dyes at concentrations up to 1.7% (as the free base).

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