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Final Report on the Safety Assessment of 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine

Animal data on 2NPPD and 4NOPD and cosmetic hair dyes containing these ingredients suggest that both compounds were nonirritating to rabbit skin and eyes, but were sensitizers on guinea pig skin. The results of repeated insult patch tests with hair dye products containing these ingredients indicated that neither was an irritant or a sensitizer to human subjects as normally used. In the absence of human data on the pure compounds, however, 2NPPD and 4NOPD are considered to be potential human sensitizers.

Topically applied 2NPPD and 4NOPD are absorbed by experimental animals. Neither embryotoxicity nor teratogenicity was observed in animal studies when hair dyes containing 2NPPD and 4NOPD were applied to the skin. Both ingredients were mutagenic in some bacterial and in vitro mammalian systems; both compounds had some genotoxic activity. In feeding studies in mice and rats, only 2NPPD induced hepatocellular tumors in female mice. Both compounds were noncarcinogenic in male mice and in rats of either sex. Epidemiological data have not demonstrated a carcinogenic effect in man for hair dyes.

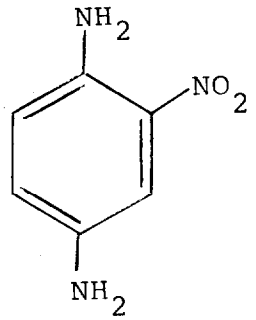
For those persons not sensitized, it is concluded that 2NPPD and 4NOPD are safe as hair dye ingredients at the current concentration of use.

INTRODUCTION

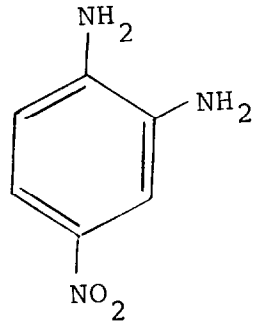
2-Nitro-*p*-phenylenediamine and 4-Nitro-*o*-phenylenediamine are reviewed in this report; they are used in both semipermanent and permanent hair dyes. *p*-Phenylenediamine is reviewed separately; it is used only in permanent hair dyes. All three compounds and other hair dye ingredients may be combined in hair dye products.

CHEMICAL AND PHYSICAL PROPERTIES

2-Nitro-*p*-phenylenediamine (2NPPD)(CAS No. 5307-14-2) and 4-Nitro-*o*-phenylenediamine (4NOPD)(CAS No. 99-56-9) are the substituted aromatic amines with the following chemical formulas[1-3]:



2-Nitro-*p*-phenylenediamine



4-Nitro-*o*-phenylenediamine

Other names for 2NPPD include: 2-nitro-1,4-benzenediamine; 2-nitro-1,4-diaminobenzene; and *o*-nitro-*p*-phenylenediamine. Other names for 4NOPD include: 4-nitro-1,2-benzenediamine; and 4-nitro-1,2-diaminobenzene.[1]

The Cosmetic, Toiletry and Fragrance Association (CTFA) Cosmetic Ingredient Chemical Description for 2NPPD is a reddish brown crystalline powder. As a solid it has good storage stability, but in solution it readily oxidizes and gives the reactive imine intermediates of oxidation dyes.[4] 2NPPD has a molecular weight of 153.14 and a melting point of 137°C. It is soluble in water and ethanol. Its color in solution depends upon the pH of the solution. 2NPPD is reddish orange in aqueous solution, and with the addition of ferric chloride it becomes black. There are IR and NMR spectra available for 2NPPD.[2,5,6]

The CTFA Cosmetic Ingredient Chemical Description for 4NOPD[7] is a red powder. In solution it readily oxidizes and gives the reactive imine intermediates of oxidation dyes. 4NOPD has a molecular weight of 153.14 and a melting point of 199 to 201°C. It is sparingly soluble in water and soluble in acetone and aqueous acids. IR and UV spectra are available for 4NOPD.[2,3,8]

2NPPD was first prepared by the hydrolysis of 1,4-diamino-4-acetyl-2-nitrobenzene. A similar method is used commercially in Japan; 2NPPD is prepared by acetylating *p*-phenylenediamine with acetic anhydride, followed by nitration and hydrolysis. A commercial grade of 2NPPD is available in the US with a minimum purity of 97.0 percent; it may contain a maximum of 100 ppm iron. 2NPPD is available in Japan with a minimum purity of 99 percent; it may contain nitroaminoacetanilide isomers as impurities.[2] Information on other impurities was not available.

4NOPD is prepared by the reduction of 2,4-dinitroaniline using hydrogen sulfide in ammonia water. A commercial grade is available in the US with a minimum purity of 98.0 percent; it may contain a maximum of 150 ppm iron.[2,3] Information on other impurities was not available.

Qualitative and quantitative determinations of 2NPPD and 4NOPD and their derivatives are made using paper chromatography,[2,9] high performance liquid

chromatography,[10] reverse-phase liquid chromatography,[11] and thin-layer chromatography[2,12-20] and by spectrophotometric methods[2,21,22] and electrophoresis.[2,14]

USE

Cosmetic

2NPPD and 4NOPD are used in semipermanent and permanent hair dye formulations.[23-26]

Semipermanent hair dye formulations are a mixture of dyes that are generally applied to the hair full strength and are left on 5 to 30 minutes before being rinsed out. Hydrogen peroxide is not used in color development, and the hair color lasts through five or six shampoos.[23,24,27,28] The dyes penetrate the cortex of the hair shaft and are fixed by oxidation by air.[23,29] 2NPPD and 4NOPD are red and yellow dyes, respectively, used in semipermanent hair dye formulations.[23,25,26]

Permanent hair dye formulations are a mixture of dyes that are mixed with hydrogen peroxide to produce oxidation products; these oxidation products couple with the unoxidized phenylenediamines and then form permanent bonds with the sulfhydryl groups present within hair shafts.[30,31] The colors are not removed by shampooing. Subsequent dyeing is necessitated by the need to color new hair growth rather than because of the fading of already colored hair.[23-27,32] 2NPPD and 4NOPD are used to produce light brown or reddish shades and medium to dark brown or reddish shades, respectively, in permanent hair dye formulations.[24-26]

Data submitted to the Food and Drug Administration (FDA) in 1981 by cosmetic firms participating in the voluntary cosmetic registration program indicated that 2NPPD and 4NOPD were used in totals of 28 and 26 hair dyes and colors, respectively, and that 4NOPD was used in 6 hair tints. 2NPPD was used in 7 hair dye and color products at a concentration of >0.1 to 1 percent and in 21 hair dye and color products at a concentration of ≤ 0.1 percent. 4NOPD was used in 12 hair dye and color products at a concentration of >0.1 to 1 percent, in 14 hair dye and color products at a concentration of ≤ 0.1 percent, and in 6 hair tints at a concentration of ≤ 0.1 percent.[33]

Voluntary filing of product formulation data with FDA by cosmetic manufacturers and formulators conforms to the prescribed format of preset concentration ranges and product categories as described in Title 21, Part 720.4 of the Code of Federal Regulations (21 CFR 720.4). Because data are only submitted within the framework of preset concentration ranges, opportunity exists for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to ten-fold error in the assumed ingredient concentration. Some cosmetic ingredients are supplied by the manufacturer at less than 100 percent concentration, and, therefore, the value reported by the cosmetic manufacturer or formulator may not necessarily reflect the actual concentration of the finished product; the actual concentration in such a case would be a fraction of that reported to the FDA.

Hair-coloring formulations containing 2NPPD and 4NOPD 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 2NPPD and 4NOPD several times a day.

Semipermanent hair dyes are usually applied in a shampoo base and contain thickeners, alkalizers, and foam stabilizers. Permanent hair dyes contain couplers and an oxidant in addition to the primary intermediate (the actual dye). Users may be exposed to reactive intermediates as well as to unreacted dyes.[23]

2NPPD and 4NOPD are "coal tar" hair dyes. They are no longer produced from coal but come from petroleum. Although the term "coal tar" is archaic, it is still used in legal documents.[34,35] Coal tar hair dyes are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal Food, Drug and Cosmetic Act of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The following caution statement should be displayed conspicuously on the labels of coal tar hair dyes:

> Caution—This product contains ingredients that may cause skin irritation on certain individuals, and a preliminary test according to accompanying directions should first be made. This product must not be used for dyeing the eyelashes or eyebrows; to do so may cause blindness.

Noncosmetic

2NPPD is used for dyeing furs.[2,25,36,37] 4NOPD is used as a reagent for the detection and determination of α-keto acids in blood and urine[2,3,26,38,39] and as a colorimetric reagent for the determination of ascorbic and dehydroascorbic acids in foods[2,40,41] and sulfur dioxide in the atmosphere.[42] 4NOPD is also used as a chelating agent for the gas chromatographic determination of selenium in biological materials.[43,44]

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Phenylenediamines have the potential to be converted, either metabolically or chemically, to compounds, such as quinones, hydroxylated or acetylated derivatives, and azo and azoxy derivatives, that may be toxicologically significant for humans and other organisms.[36]

2NPPD(^{14}C) was administered intraperitoneally in a dose of 2.6 mg/kg to rats, and the tissue distribution of the radioactivity was determined over a 48-hour period. The concentrations of radioactivity in all the tissues examined (blood, brain, lung, liver, kidney, adrenal, testicle, muscle, stomach, small intestine, large intestine, heart, spleen, pancreas, epididymis, seminal vesicle, and prostate), except the large and small intestines, were greatest 1 hour after dosing. The concentrations of radioactivity in the large and small intestines peaked at 6 hours and 3 hours, respectively. Highest concentrations of radioactivity were observed in the large and small intestines (including their contents), liver, kidney, and adrenal, and lowest concentrations were observed in the brain and testicle. The concentrations of radioactivity in the lung, heart, spleen, pancreas, epididymis, seminal vesicle, and prostate were similar to that in the blood. The greatest percentages of administered radioactivity were found in the muscle, small and large

intestines, and liver. By 48 hours postdosing, most of the radioactivity had disappeared from the tissues. The same dose was administered intraperitoneally to rats and excretion was monitored. Within 24 hours, almost 92 percent of the radioactivity was excreted; 37.4 percent was in the urine and 54.3 percent was in the feces. After 4 days, 96 percent of the radioactivity had been excreted. Within 24 hours of intravenous administration of 2.6 mg/kg labeled 2NPPD to cannulated rats, 42.2 percent of administered radioactivity was excreted in the bile and 34.5 percent was observed in the urine, 8.1 percent was observed in the feces, and 0.65 percent of the radioactivity was observed in the digestive tract (including its contents). Four metabolites and nonmetabolized 2NPPD were detected by thin-layer chromatography in rat urine collected for 24 hours after the intraperitoneal administration of 100 mg/kg 2NPPD. Two of the metabolites were not identified. The other two were N^4-acetyl-2-nitro-*p*-phenylenediamine and N^1, N^4-diacetyl-2-amino-*p*-phenylenediamine.[45]

2NPPD (^{14}C, uniformly labeled) in acetone was applied to the ventral forearms of adult male and female rhesus monkeys and to the backs of immature male and female Pitman-Moore white swine.[32] Groups of 3 to 6 animals were used. The 2NPPD was applied in a dose of 4 $\mu g/cm^2$ to a skin-contact area ranging from 3 to 15 cm^2. The chemical was applied to clipped skin, the skin was left uncovered, and after 24 hours, the skin was washed with soap and water. Urine was collected over a 5-day period, and the amount of radioactivity found in the urine was used to estimate the fraction of 2NPPD that penetrated the skin. A correction factor to account for the radioactivity remaining in the body during the 5 days was obtained by determining the 5-day urinary excretion of radioactivity following an intravenous or subcutaneous injection of a known amount of labeled 2NPPD. The peak rate of urinary excretion of radioactivity for monkeys was 4 to 8 percent/hour and for pigs was 8 to 12 percent/hour. It was estimated that 29.9 percent of the applied 2NPPD penetrated the skin of monkeys and 17.7 percent of the applied 2NPPD penetrated the skin of pigs during the 24-hour exposure period.

Pairs of male and female rats were intubated or injected intraperitoneally with 0.5 ml of 2NPPD (^{14}C) in 5 percent Tween 80. Expired air was assayed for radioactivity for 24 hours, and urine and feces were assayed for 3 days. At 3 days, the rats were killed and the carcasses were assayed. No radioactivity was detected in the expired air. At 3 days, 1 to 2 percent of the applied radioactivity was in the carcass. The rate of excretion was rapid; 85 to 90 percent of the applied radioactivity was recovered in the urine and feces within 24 hours. Over 3 days, 48 to 68 percent was recovered in the feces and 27 to 41 percent in the urine. There were six metabolites and a trace of unchanged 2NPPD in the urine; the metabolites were acetylated 2NPPD, sulfate and/or glucuronide conjugates of 2NPPD and of acetylated 2NPPD, and two conjugates with sulfur-containing amino acids. Similar metabolites were detected in the feces.[46]

The backs of 6 male and 6 female rats were clipped, and 100 or 200 μl of 2NPPD (^{14}C) in ethanol was applied to a 10 cm^2 area. The application site was allowed to dry and was covered with a protective patch. Excreta was collected at 24 and 48 hours. At 48 hours, the animals were killed, and the excreta, carcasses, skin, and patches were assayed for radioactivity. Skin penetration was calculated by adding the determinations for the carcasses and excreta. The female rats absorbed 29 percent of the applied radioactivity; at 48 hours, 40 percent of that ab-

sorbed was in the urine, 53 percent was in the feces, and 7 percent was in the carcasses. Male rats absorbed 14 percent of the applied radioactivity; at 48 hours, 34 percent of that absorbed was in the urine, 61 percent was in the feces, and 5 percent was in the carcasses. The same pattern of distribution of radioactivity among metabolites was observed as in the orally or parenterally dosed animals.[46]

The same researchers conducted several similar experiments with a hair colorant base containing 2NPPD.[46] Groups of 3 female rats were treated with 100 μl of 50 percent aqueous hair colorant base containing 0.5 percent 2NPPD (^{14}C) on 10 cm^2 of skin for 5 minutes. One group was killed immediately after rinsing with distilled water, one group each was rinsed or not rinsed and a nonocclusive 48-hour patch was applied, and one group each was rinsed or not rinsed and an occlusive 48-hour patch was applied. Excreta, carcasses, skin, and rinsings were assayed for radioactivity, and skin penetration (excreta plus carcasses) was calculated. Skin penetration was 1.7 $\mu g/cm^2$ in the rinsed, nonocclusive patch group, 5.0 $\mu g/cm^2$ in the not rinsed, nonocclusive patch group, 4.2 $\mu g/cm^2$ in the rinsed, occlusive patch group, and 33.9 $\mu g/cm^2$ in the not rinsed, occlusive patch group. Groups of 3 female rats were treated with 200 μl of 50 percent aqueous hair colorant base containing 0.6 percent 2NPPD (^{14}C) on 10 cm^2 of skin for 5 to 30 minutes before rinsing. After rinsing, the skin was covered with a nonocclusive patch. At 48 hours, skin penetration had increased from 3.2 $\mu g/cm^2$ for a 5-minute contact to 6.1 $\mu g/cm^2$ for a 30-minute contact. Three groups of 4 female rats were treated with 200 μl of a 50 percent aqueous hair colorant base containing 0.5 percent 2NPPD (^{14}C) on 10 cm^2 of skin for 10 minutes and were rinsed. A further application was made to one group an hour later, and two further applications were made to another group at hourly intervals. After final treatment, a 48-hour nonocclusive patch was applied. Skin penetration was 4.8 $\mu g/cm^2$ in the single application group, 13.2 $\mu g/cm^2$ in the double application group, and 13.7 $\mu g/cm^2$ in the triple application group.

Another experiment was conducted with groups of 2 to 3 rats. Each rat was treated with 200 μl of 50 percent hair colorant base containing 0.025 to 0.48 percent 2NPPD (^{14}C) on 10 cm^2 of skin for 5 minutes. The base was rinsed off, and a 48-hour nonocclusive patch was applied. Skin penetration increased with increasing 2NPPD concentration and was proportional to 2NPPD concentration; it was 0.1 $\mu g/cm^2$ with 0.025 percent 2NPPD and 3.2 percent with 0.48 percent 2NPPD. In another experiment, a tuft of hair was left on the backs of some of the rats and 200 μl of 50 percent shampoo base containing 0.5 percent 2NPPD (^{14}C) was applied onto 10 cm^2 of skin for 5 minutes. The skin was rinsed and protected with a nonocclusive patch. The hair was clipped 48 hours after base application, and it contained 28 percent of the applied radioactivity; skin penetration and radioactivity in rinsings were both reduced by hair. Clipped skin penetration was 3.2 $\mu g/cm^2$ and hairy skin penetration was 1.6 $\mu g/cm^2$.

A composite of semipermanent hair dyes and base components (15 dyes and 10 base components) containing 0.24 percent 2NPPD and 0.16 percent 4NOPD was given to dogs in doses of 19.5 and 97.5 mg/kg per day in their feed, to rats in concentrations of 1950 and 7800 ppm in their feed, and to rabbits in doses of 19.5 and 97.5 mg/kg per day in a 0.5 percent aqueous methylcellulose vehicle by gavage.[47] All the animals excreted blue-brown colored urine daily. The urine was much the same in color as that obtained by adding the composite to urine.

The urine collected from the dogs following overnight fasting and the urine collected from the rabbits each day prior to dosing was normal in color. The researchers stated that this was probably an indication of rapid clearance.

2NPPD and 4NOPD were mutagenic in the Ames test[48] without metabolic activation for *Salmonella typhimurium* strain TA1538.[49] Five mg of 4NOPD and 0.5 ml of a permanent hair dye containing 4NOPD (0.5 mg) were injected intraperitoneally into rats. Only 1 rat survived for 24 hours after the hair dye injection. The urine from the rat was assayed for mutagenicity using the method of Durston and Ames.[50] One to 1.5 percent of the injected 4NOPD appeared in the urine in a form directly mutagenic to *S. typhimurium* strain TA1538. Urine from the rat injected with the hair dye had mutagenic activity in proportion to the amount of 4NOPD contained in the product.

4NOPD was applied topically in a dose of 120 mg in acetone and in isopropanol for 20 minutes to shortened hair on the backs of rats.[49] Then, the rats were shampooed and rinsed. Urine specimens were collected before dye application and daily for 4 days; the specimens were assayed for mutagenicity using *S. typhimurium* strain TA1538. Mutagenic activity was observed in the urine collected following 4NOPD application to the backs of rats. More activity was observed when the 4NOPD was applied in acetone than when it was applied in isopropanol. The researchers commented that the isopropanol vehicle probably more closely approximated actual use conditions, since isopropanol is a base ingredient in hair-coloring products. The same procedures were used with three permanent hair dyes: dye A contained 2.5 mg of 4NOPD/ml, dye B contained 1 mg of 4NOPD/ml, dyes A and B also contained 2NPPD, and dye C contained neither 4NOPD nor 2NPPD. Dyes A and B were both mutagenic, but mutagenic activity was greater for dye A. Maximal mutagenic activity was observed in the urine collected during the first 24 hours following dye application. No significant mutagenic activity was observed in the urine collected 2 to 4 days after dye application. Mutagenic activity may not have been due solely to 4NOPD. Addition of an equal volume of hydrogen peroxide to the dyes prior to application did not have a consistent effect on mutagenic activity; mutagenicity was decreased for dye A and increased for dye B. The urine of rats to which dye C was applied was negative in this test system. Urine collected before dye application had no mutagenic activity.

ANIMAL TOXICOLOGY

Oral Studies

Acute Toxicity

The acute oral toxicity of 2NPPD and 4NOPD was studied in rats and mice (Table 1).[2,51-53] The LD_{50} values obtained for 2NPPD for rats varied from 1800 to 3080 mg/kg and for 4NOPD varied from 681 to 3720 mg/kg. In the Hodge and Sterner[54] classification of single-dose oral toxicity for rats, 2NPPD and 4NOPD would be classified as slightly toxic.

Subchronic and Chronic Toxicity

The subchronic and chronic oral toxicity of 2NPPD, 4NOPD, and a composite of semipermanent hair-coloring ingredients (containing 2NPPD and 4NOPD)

TABLE 1. Acute Oral Toxicity of 2NPPD and 4NOPD

Material Tested	*No. and Species of Animals*	*LD_{50} (mg/kg)*	*Comments*	*Reference*
2NPPD in water	Male rats	2100	–	2,52
2NPPD in oil-in-water emulsion	5 male and 5 female rats at each dose	3080	–	51
2NPPD in 0.5 percent aq. gum tragacanth (with 0.05 percent Na_2SO_3 and adjusted to pH 7.0)	5 male and 5 female rats at each dose	1800	Lethargy and piloerection after dosing. Red-stained urine. Red-stained internal organs. No other abnormalities at autopsy	53
4NOPD	Rats	681	–	56
	Mice	681		
4NOPD in oil-in-water emulsion	5 male and 5 female rats at each dose	3720	–	51
4NOPD in 0.5 percent aq. gum tragacanth (with 0.05 percent Na_2SO_3 and adjusted to pH 7.0)	5 male and 5 female rats at each dose	2100	Lethargy and piloerection after dosing. Orange-stained urine. No abnormalities at autopsy	53

was studied in rats, mice, rabbits, and dogs (Table 2).[2,25,26,47,52,55] Dietary concentrations for 4 weeks of up to 6800 ppm 2NPPD for rats and up to 11,830 ppm 2NPPD for mice were nontoxic. Dietary concentrations for 78 weeks of up to 1100 ppm 2NPPD for rats and up to 4400 ppm for mice resulted in reduced mean body weights but no other signs of toxicity. Rats fed diets for 7 weeks containing concentrations of 6800 to 10,000 ppm 4NOPD had arched backs and rough coats, and mice fed diets containing concentrations of 14,700 to 21,500 ppm 4NOPD had orange-colored fur. Dietary concentrations of up to 750 ppm 4NOPD for 102 to 103 weeks in rats were nontoxic. Dietary concentrations of up to 7500 ppm 4NOPD for 102 to 103 weeks in mice resulted in reduced body weights but no other signs of toxicity. A hair-coloring composite containing 0.24 percent 2NPPD and 0.16 percent 4NOPD at concentrations up to 7800 ppm was fed to male and female rats for more than 8 weeks and to pregnant rats for 9 days during gestation. The composite was also fed to dogs in doses of up to 97.5 mg/kg per day for 2 years and was administered by gavage in doses of 97.5 mg/kg per day to pregnant rabbits for 12 days during gestation. No adverse effects were observed; all the animals excreted blue-brown urine.

Dermal Studies

Acute Toxicity

Groups of 4 to 8 rabbits were shaved (area of application unspecified), and 2NPPD and 4NOPD in 10 ml of a permanent hair dye base were applied topically for 24 hours. No deaths occurred at doses of 5 g/kg of 2NPPD and 4NOPD.[51]

Subchronic Toxicity

A hair dye composite containing 0.55 percent 2NPPD was diluted 1:1 with a hydrogen peroxide activator and applied in doses of 1.0, 2.0, and 4.0 g/kg per day for 20 days to the shaved backs of groups of male and female rabbits (number unspecified). The application site was approximately 10 percent of the body surface. There was also a group of untreated control animals. The skin of 2 animals from each group was abraded prior to composite application. The rabbits were observed for a further 14 days after the test period. Two rabbits died during the test period in the 1.0 g/kg group, 2 died in the 2.0 g/kg group, 1 died in the 4.0 g/kg dye-treated group, and 1 died in the control group. These deaths were attributed to naturally occurring disease; the incidence and severity of disease may have been increased due to the stress of the severe local skin reactions and the dosing procedure. There were adverse effects on body weight in the treated rabbits during the test period, but body weights were comparable to the controls during the observation period. There were no significant adverse findings in the hematological and clinical chemistry parameters or in the urinalyses. No clinical signs of toxicity were observed. From Day 5 to Day 20 of the test period, local skin reactions were characterized by escharosis, with subsequent sloughing of the skin at the application site in the treated animals. By the end of the 14-day observation period, the skin appeared normal. No significant gross or microscopic alterations were observed in the tissues and organs of the rabbits killed at the end of the study or in any treated animals that died during the study, except for the skin. At the dye application site in a few animals, edema and/or hyperkeratosis was observed.[57]

A hair dye composite containing 0.55 percent 2NPPD was diluted 1:1 with a hydrogen peroxide activator, and 2 ounces of the mixture were distributed throughout the hair and allowed to contact the hair and skin of male and female rabbits (unspecified number) for 30 minutes. The rabbits' hair was then rinsed with tap water. This procedure was repeated once every 2 weeks for 13 weeks, for a total of seven applications. No significant adverse findings were noted in mortality, behavior, local skin reactions, body weights, hematological and clinical chemistry values, urinalyses, gross and microscopic pathological studies, and organ weights.[58]

A composite hair dye formulation containing 0.013 percent 4NOPD was mixed 1:1 with a peroxide formula, and two groups of 10 rabbits (5 male and 5 female) received percutaneous doses of 1.0 and 4.0 g/kg per day of the mixture for 20 days. The application site was the shaved back, and it comprised approximately 10 percent of the body surface. The 20-day test period was followed by a 14-day observation period. No rabbits died, and there were no signs of systemic toxicity except for lassitude and reduced feed intake. By the end of the observation period, these signs had disappeared; all animals appeared normal and there was a net body weight gain. Local skin reactions were noted in both test groups; erythema was noted on Day 2; on Day 6 some rabbits in both groups had pinpoint intradermal or subdermal hemorrhages; on Day 7 or 8, the skin was dried and wrinkled, and this was followed by desquamation. Hair regrowth was retarded during the 20-day test period. At the end of the observation period, hair and skin appeared normal. No significant gross abnormalities were noted at necropsy.[59]

TABLE 2. Subchronic and Chronic Toxicity of 2NPPD and 4NOPD

Material Tested	*Dose and Vehicle*	*Length of Study*	*No. and Species of Animals*	*Results*	*Reference*
2NPPD	0–6,800 ppm in diet (6 doses)	4 weeks (2 weeks further observation)	5 male and 5 female rats per dose	No abnormal clinical signs recorded	25,55
2NPPD	0–11,830 ppm in diet (9 doses)	4 weeks (2 weeks further observation)	5 male and 5 female mice per dose; 2 control groups	No abnormal clinical signs recorded	25,55
2NPPD	Diet contained 500 mg/kg of 2NPPD	13 weeks	10 male and 10 female rats	No changes in body weight, blood or urine parameters, or histological appearance of a range of tissues (vs. controls)	2,52
2NPPD	0–1,100 ppm male rats in diet; 0–2,200 ppm female rats in diet (3 doses)	78 weeks (27 weeks further observation)	50 male or 50 female rats per dose (20 of each sex as controls)	No significant association with mortality of either sex. Mean body weight depression observed for both sexes, relative to controls; concentrations may have approximated maximum tolerated dosages. No other abnormal clinical signs recorded	25,55
2NPPD	0–4,400 ppm in diet (3 doses)	78 weeks (12 to 13 weeks further observation)	50 male and 50 female mice per dose (20 of each sex as controls)	No significant association with mortality of either sex. Mean body weight depression observed for both sexes, relative to controls; concentrations may have approximated maximum tolerated dosages. No other abnormal clinical signs recorded	25,55
4NOPD	0–10,000 ppm in diet (9 doses)	7 weeks (1 week further observation)	5 male and 5 female rats per dose, 2 control groups	Arched backs and rough coats at 2 highest doses (6,800 and 10,000 ppm)	26
4NOPD	0–21,500 ppm in diet (9 doses)	7 weeks (1 week further observation)	5 male and 5 female mice per dose; 2 control groups	Orange-colored fur at 2 highest doses (14,700 and 21,500 ppm)	26
4NOPD	0–750 ppm in diet (3 doses)	103 weeks (2 weeks further observation)	50 male and 50 female rats per dose (20 of each sex as controls)	No "distinct" mean body weight depression, no significant increase in mortality, no other signs of chronic toxicity, no clinical signs recorded; possibly rats could tolerate higher dietary concentration	26

4NOPD	0–7,500 ppm in diet (3 doses)	102 weeks (2 weeks further observation)	50 male and 50 female mice per dose (20 of each sex as controls)	"Distinct" dose-related mean weight depression; concentrations may have approximated maximum tolerated dosages; no other clinical signs recorded	26
Composite of semi-permanent hair-coloring ingredients (0.24 percent 2NPPD and 0.16 percent 4NOPD)	0–7,800 ppm in diet (3 doses)	9 days (Days 6 to 15 of gestation)	20 pregnant female rats per dose	No adverse effects. Excreted urine blue-brown in color	47
Composite of semi-permanent hair-coloring ingredients (0.24 percent 2NPPD and 0.16 percent 4NOPD)	0–97.5 mg/kg per day in 0.5 percent aq. methylcellulose (by gavage) (3 doses)	12 days (Days 6 to 18 of gestation)	12 pregnant female rabbits per dose	No adverse effects. Excreted urine blue-brown in color after dosing. Normal in color each day previous to dosing	47
Composite of semi-permanent hair-coloring ingredients (0.24 percent 2NPPD and 0.16 percent 4NOPD)	0. to 7,800 ppm in diet (3 doses)	>8 weeks	10 male and 20 female rats per dose	No effects on body weight gain or food consumption. Excreted urine blue-brown in color	47
Composite of semi-permanent hair-coloring ingredients (0.24 percent 2NPPD and 0.16 percent 4NOPD)	0 to 97.5 mg/kg per day in diet (3 doses)	2 years	6 male and 6 female dogs per dose	No deaths. No adverse effects on weight gain or clinical, hematological, blood chemical, and urinalysis parameters. Excreted blue-brown urine except after overnight fasting	47

Two groups of 10 rabbits (5 male and 5 female) received cutaneous applications of 1.0 and 4.0 g/kg per day for 20 days of a composite hair dye formulation containing 0.036 percent 4NOPD that was mixed with creamy peroxide activator before use. The hair dye was applied to the shaved back (approximately 10 percent of the body surface). The animals were observed for 14 days after the test period. Two rabbits in the 4.0 g/kg per day group died during the study; these deaths were ascribed to severe diarrhea, which was not considered related to the application of the composite. Lassitude and reduced feed intake were noted during the test period. These disappeared over the observation period, and a net body weight gain was observed. Local skin reactions included erythema at Day 2, pinpoint subdermal hemorrhages at Day 7, and drying and wrinkling at Day 8, followed by desquamation. Hair regrowth was retarded during the test period. At the end of the observation period, skin and hair appeared normal. No gross lesions were found at necropsy except for enterocolitis in the 2 animals that died during the test period; this was consistent with the observation of severe diarrhea.[60]

Two permanent hair dye formulations, one containing 1.1 percent 2NPPD and one containing 0.25 percent 4NOPD, were mixed with an equal volume of 6 percent hydrogen peroxide. Two milliliters per kilogram of each hair dye was applied topically every third day of gestation for 19 days to a group of 20 pregnant rats. No signs of toxicity were observed. Maternal weight gain and feed consumption were similar to those of the controls. There was a change in the color of the hair and skin at the site of application, but no irritation was observed.[61]

These same hair dye formulations were mixed with an equal volume of 6 percent hydrogen peroxide and applied topically two times a week for 13 weeks to groups of 6 female and 6 male rabbits. A 1 ml/kg dose of 1:1 dye:hydrogen peroxide was applied to alternating sites. The application sites of half of the animals were abraded once each week. The rabbits were restrained for an hour after application and were shampooed, rinsed, and dried. There were no clinical signs of compound-induced toxicity. Body weight gains were equal to those of the controls. Urinalysis findings were "unremarkable," and the urine was not discolored. No gross or microscopic lesions related to dye application were seen, and no significant differences were observed in clinical chemistry or hematological values.[61]

Chronic Toxicity

A semipermanent hair colorant shampoo containing unspecified concentrations of 2NPPD and 4NOPD was diluted 1:4:5 with water and acetone. A 0.4 ml dose of the mixture was usually applied twice a week to the clipped backs of groups of 16 to 26 male and female A and DBAf strains of mice. At 24 weeks, the volume was reduced to 0.2 ml for the DBAf mice. The experimental period was 80 weeks, and a total of 138 applications were made. The treatment was well tolerated by the A mice and initially was well tolerated by the DBAf mice. Between 13 and 24 weeks, some male DBAf mice became emaciated and the volume applied was halved as a result. Toxic effects were mainly in the urogenital tract and may have been due to obstructing crystals that were sometimes observed in the urinary bladder and on the skin around the penis. The preputial region was frequently distended. The urinary bladder and seminal vesicles were distended and the renal tubules were dilated. Many of the DBAf mice had noticeably distended stomachs; 3 of 32 controls and 4 of 41 treated mice had chronic gastritis.[62]

Two hair dye composite formulations, one containing 1.1 percent 2NPPD and one containing 0.25 percent 4NOPD, were mixed 1:1 with 6 percent hydrogen peroxide, and 0.05 ml of the mixture was applied once a week to the clipped intrascapular region of groups of 50 male and 50 female mice for 21 to 23 months. There were three shaved but untreated control groups. At 7 and 9 months, 20 mice from each group were killed and necropsied. No differences were observed in body weights, mean absolute and relative weights of the liver and kidneys, and survival rates.[63]

Hair dye composite formulations containing 1.1 percent 2NPPD or 0.25 percent 4NOPD were mixed 1:1 with 6 percent hydrogen peroxide and applied topically to a rat F_0 generation from the time of weaning to the weaning of the F_{1A} generation. Groups of 60 male and 60 female rats of the F_{1A} generation received 0.2 ml of the hair dye (mixed 1:1 with 6 percent hydrogen peroxide), which was increased by 0.1 ml weekly to 0.5 ml, two times a week for 2 years on the clipped neck and back. Ten rats from each group were killed and necropsied at 12 months. There were three clipped but untreated control groups. Dry skin was observed in the first few weeks of the study in 15 to 20 percent of the female rats, and slightly decreased mean values for total erythrocytes, hemoglobin, and packed cell volume were observed in the male rats receiving the hair dye containing 1.1 percent 2NPPD. No other differences were observed in general behavior, appearance, or in clinical chemistry or urinalyses findings.[64]

Primary Skin Irritation

The primary skin irritation of 2NPPD and 4NOPD was studied in rabbits (Table 3).[53,65] The Primary Irritation Index (PII) for a 2.5 percent concentration of 2NPPD in aqueous gum tragacanth was 0, for a 2.5 percent concentration of 4NOPD in aqueous gum tragacanth was 0, and for a 5.0 percent concentration of 4NOPD in ethanol was 0.38. Both compounds were nonirritating to rabbit skin.

Skin Sensitization

Two groups of 20 guinea pigs received topical applications of 3 percent 2NPPD or 4NOPD in an aqueous solution containing 2 percent Natrosol 250 HR, 2 percent Tween 80, 0.05 percent sodium sulfite, and 10 percent isopropanol, and adjusted to pH 7. The compounds were applied daily 6 days a week for 3 weeks on a 6 cm^2 shaved area of the flank. A 2-week rest period was followed by application of the compounds on the opposite, previously untreated flanks of the guinea pigs. Four of 20 guinea pigs were sensitized to 2NPPD; the total reaction intensity for all the animals was 4 (scores added for all animals; possible total of 60). Eighteen of 20 guinea pigs were sensitized to 4NOPD; the total reaction intensity was 20 (possible total of 60). The researchers stated that 2NPPD produced a "weak reaction" and that 4NOPD produced a "relatively strong reaction."[66]

It was reported that 1 in 10 guinea pigs previously sensitized to *p*-phenylenediamine was also sensitive to 4NOPD.[65] The Cosmetic Ingredient Review (CIR) Expert Panel is currently reviewing *p*-phenylenediamine.

Eye Irritation

The ocular irritation of 2NPPD and 4NOPD was studied in rabbits (Table 4).[53,65] Concentrations of 2.5 percent 2NPPD and 4NOPD in aqueous gum tragacanth were instilled into the eyes of rabbits; occasional, mild conjunctival irrita-

TABLE 3. Primary Skin Irritation of 2NPPD and 4NOPD

Material Tested	*Concentration (percent)*	*Method*	*No. of Rabbits*	*Results*	*Reference*
2NPPD in 0.5 percent aq. gum tragacanth (with 0.05 percent Na_2SO_3 and adjusted to pH 7.0)	2.5 (w/v)	Based on Code of Federal Regulations (CFR), Title 16, Sec. 1500.41 (total possible PII = 8)	3	PII = 0	53
4NOPD in 0.5 percent aq. gum tragacanth (with 0.05 percent Na_2SO_3 and adjusted to pH 7.0)	2.5 (w/v)	Based on 16 CFR 1500.41 (total possible PII = 8)	3	PII = 0	53
4NOPD in ethanol	5	Ref. 66	–	PII = 0.38	65

TABLE 4. Eye Irritation by 2NPPD and 4NOPD

Material Tested	*Concentration (percent)*	*Method*	*No. of Rabbits*	*Results*	*Reference*
2NPPD in 0.5 percent aq. gum tragacanth (with 0.05 percent Na_2SO_3 and adjusted to pH 7.0)	2.5 (w/v)	Instilled into one eye. Irrigated with 50 ml of lukewarm water (37°C) 10 seconds after instillation	3	Occasional transient mild conjunctival inflammation. Did not persist more than 24 hours	53
4NOPD in 0.5 percent aq. gum tragacanth (with 0.05 percent Na_2SO_3 and adjusted to pH 7.0)	2.5 (w/v)	Instilled into one eye. Irrigated with 50 ml of lukewarm water (37°C) 10 seconds after instillation	3	Occasional transient mild conjunctival inflammation. Did not persist more than 24 hours	53
4NOPD	100	Ref. 66 Possible score = 110	–	Score = 3.0	65

tion that did not persist more than 24 hours was observed. A 100 percent concentration of 4NOPD was also instilled into the eyes of rabbits; the irritation score was 3.0 (possible maximum of 110). Both 2NPPD and 4NOPD were nonirritating to the rabbit eye.

Other Studies

2NPPD and 4NOPD in 10 percent aqueous dimethyl sulfoxide (DMSO) were administered intraperitoneally to groups of 10 male rats. The acute intraperito-

neal LD_{50}s of 2NPPD and 4NOPD to rats were 348 mg/kg and greater than 1600 mg/kg, respectively.[51]

Groups of 3 rats were given 40 mg/kg per day of 2NPPD in DMSO or 100 mg/kg per day of 4NOPD in DMSO intraperitoneally for 5 days and were observed for 7 days following the first injection. Both compounds reduced body weight gains but were not lethal.[67,68]

Aqueous solutions of 2NPPD and 4NOPD were administered intraperitoneally three times weekly for 8 weeks to groups of 20 male rats in doses of 20 mg/kg. Forty controls received sterile water. The treated rats gained 15 percent less weight than the controls did over the 8-week period.[51]

Aqueous solutions of 2NPPD and 4NOPD were administered subcutaneously to pregnant mice on Days 6 to 15 of gestation. Doses of 2NPPD of 32 mg/kg per day to greater than 160 mg/kg per day and of 4NOPD equal to or greater than 256 mg/kg per day significantly reduced maternal weight gain. Significant maternal toxicity was observed at doses of 2NPPD of 160 mg/kg per day or more.[69,70]

SPECIAL STUDIES

Animal Reproduction and Teratology

Aqueous solutions of 2NPPD and 4NOPD were administered subcutaneously to pregnant mice on Days 6 to 15 of gestation. The mice were killed on Day 18 and the fetuses were examined. Doses of 2NPPD equal to or greater than 160 mg/kg per day produced significant maternal toxicity and significantly increased the number of fetuses with cleft palates and major blood vessel malformations. Dose-related increases in the incidences of resorptions and stunted fetuses were observed. Average fetal weight was lower at doses of 128 mg/kg per day or greater, and maternal weight gain was significantly decreased at doses of 32 and 128 to 256 mg/kg per day. The highest "no effect" dose of 2NPPD was 64 mg/kg per day. Doses of 4NOPD of 256 mg/kg per day or greater significantly increased the incidence of fetuses with cleft palates and major blood vessel malformations and significantly decreased average fetal weight and maternal weight gain. These changes were not accompanied by dose-related increases in resorptions or fetal deaths. White spots that stained red with alizarin red S were seen in a significant number of left cardiac ventricles in fetuses exposed to the higher doses of both 2NPPD and 4NOPD.[69,70]

Two permanent hair dyes, one containing 1.1 percent 2NPPD and one containing 0.25 percent 4NOPD, were applied to the skin of groups of 20 pregnant rats every 3 days from Day 1 to 19 of gestation. The hair dyes were applied in doses of 2 ml/kg and were mixed with equal volumes of 6 percent hydrogen peroxide prior to use. There were three negative (untreated) and one positive control groups. The hair dyes had no embryotoxic or teratogenic effects. There were no biologically significant soft tissue or skeletal changes in the fetuses. The mean numbers of corpora lutea, implantation sites, live fetuses, resorptions per pregnancy and litters with resorptions, and the sex ratio were not significantly affected by hair dye treatment. No signs of maternal toxicity were observed. Female body weights and feed consumption of test rats were similar to the negative controls.[61]

Hair dye formulations containing 1.1 percent 2NPPD and 0.25 percent 4NOPD were mixed with 6 percent hydrogen peroxide and applied two times a week to the clipped back and necks of groups of 40 male and 40 female rats (the

F_0 generation). The initial dose was 0.2 ml of the dye per application, and the dose was increased by 0.1 ml/application weekly to a dose of 0.5 ml/application. Treatment was continuous through growth, mating, gestation, and lactation to the weaning of the F_{1B}, F_{2B}, and F_{3C} litters of the respective generations. There were three clipped but untreated control groups. The dye-treated groups were comparable to the control groups in general behavior and appearance, feed consumption, body weight gain, and survival. Treated rats did have a few skin reactions throughout the study; these included mild scabbing, fissuring, atonia, and leathery texture. The treated F_0, F_1, and F_2 parents did not differ from the controls in fertility, gestation, survival, and live birth indices. Litter size and body weights of the young were similar. There were no treatment-related gross or microscopic lesions observed in the F_{1B} parental rats or F_{3B} weanling rats killed and necropsied during the study. There were no treatment-related gross lesions observed in the rats that died during the study.[71]

A hair dye formulation containing 1.1 percent 2NPPD and 0.25 percent 4NOPD was applied topically in a dose of 0.05 ml to the clipped backs of 50 female mice two times a week for 4 weeks prior to mating and throughout mating and gestation. Evidence of mating was observed in 33 mice, and 26 of those became pregnant. Of the 50 clipped but untreated control mice, evidence of mating was found in 30, and 23 of these became pregnant. No signs of maternal toxicity were observed. The maternal weight gains and pregnancy and mortality rates of the treated mice were comparable to the controls. The mean numbers of implantations, live fetuses, and resorptions and fetal sex ratios and numbers of skeletal and soft tissue malformations were similar in treated and control mice. Slightly lower (not statistically significant) fetal weights were observed in the treated mice, but the mean crown-rump distances were comparable to the controls. The researchers concluded that there was no evidence of a teratogenic effect. However, there may have been a retarding effect on the ossification process, particularly of the bones of the feet and of the cervical and caudal vertebral centra.[72]

The same hair dye formulation (1.1 percent 2NPPD and 0.25 percent 4NOPD) was applied topically in a dose of 2 ml/kg to the clipped backs of more than 30 female rabbits two times a week for 4 weeks prior to mating and throughout mating and gestation. Thirty of the rabbits were mated, 21 became pregnant, and 4 of those mated died. (Thirty-two untreated control rabbits were mated, 21 became pregnant, and 6 of those mated died.) No signs of maternal toxicity were observed. There were no adverse effects on pregnancy rates and maternal survival and body weights. Focal alopecia was noted at slightly higher incidences in treated rabbits during the first two thirds of gestation; in the last third of gestation, the incidence of alopecia in control and treated rabbits was similar. The mean numbers of corpora lutea, implantations, and live fetuses, implantation efficiency, and number of doses with two or more resorptions were comparable in control and treated rabbits. There was no evidence of a teratogenic effect. Embryotoxicity may have occurred, as the percent of live fetuses was significantly less in the treated rabbits (85.9 percent in the treated rabbits and 93.8 percent in the control rabbits), and the percent of resorbed fetuses was significantly greater (14.1 percent in the treated rabbits and 6.2 percent in the control rabbits).[73]

A semipermanent hair-coloring composite containing 0.24 percent 2NPPD and 0.16 percent 4NOPD was administered to groups of 10 male and 20 female

rats in their diets in concentrations of 0, 1950, and 7800 ppm. In the first study, males were fed the test diets 8 weeks prior to mating and during mating, and females were fed the basal diet. In the second study, females were fed the test diet 8 weeks prior to mating and during gestation and 21 days of lactation, and males were fed the basal diet. In both studies, no dose-related significant differences were observed in male and female fertility, length of gestation, number of females with absorption sites, live pups per litter, pup body weights, and pup survival. There were no abnormal pups. No effects on feed consumption or body weight gains of either sex were found. In a third study, the composite was administered in the diet in the same concentrations as in the first two studies to groups of 20 pregnant rats on Days 6 to 15 of gestation. The rats were killed on Day 19. The composite had no adverse effects on pregnant rats or pups. No dose-related significant differences were found in the average numbers of implantation sites, live pups, and early or late resorptions per litter, and the number of females with one or more resorption sites. One of 244 pups was grossly abnormal in the 0 ppm group, none of 244 were grossly abnormal in the 1950 ppm group, and 1 of 262 was grossly abnormal in the 7800 ppm composite dietary group. The litter in the high-dose group with an abnormal pup also included 13 normal pups.[47]

Groups of 12 pregnant rabbits were dosed by gavage on Days 6 to 18 of gestation with 0 (received the 0.5 percent methylcellulose vehicle), 19.5, and 97.5 mg/kg of the same semipermanent hair-coloring composite. They were killed on the thirtieth day of gestation. No evidence of teratogenic effects was found. Fetal survival was not affected and no abnormal fetuses or soft tissue defects were observed.[47]

Short-Term Predictive Tests for Mutagens and Carcinogens

Mutations

Bacteria

2NPPD and 4NOPD were mutagenic for some strains of *S. typhimurium* in the Ames test[48] with and without metabolic activation[49,74-89] (Table 5).

A workshop held under the auspices of the National Institute of Environmental Health Sciences discussed the protocol of the Ames assay and recommended that 4NOPD be used as a positive control for *S. typhimurium* strains TA1538 and TA98 without metabolic activation.[90] 4NOPD does appear as a positive control in the literature.[91-95]

Zeiger et al.[96] suggested that filter paper discs impregnated with 4NOPD be used in a scheme to confirm the phenotype of the standard set of *S. typhimurium* tester strains. 4NOPD was positive for strains TA1538, TA98, and TA100 and was negative for strains TA1535 and TA1537.

Several hair dyes containing 2NPPD and 4NOPD were mutagenic in the Ames test for *S. typhimurium* strains TA1538 and TA98 with and without metabolic activation and negative for strain TA1535 with and without metabolic activation.[89,97,98]

Mutagenic activity using *S. typhimurium* strain TA1538 was detected in the urine of rats after the intraperitoneal injection of 4NOPD or a complete dye formulation containing 4NOPD and after the topical application of 4NOPD or two commercial, oxidative-type hair dyes containing 2NPPD and 4NOPD. Topically

TABLE 5. Mutagenicity to *Salmonella typhimurium*

Material Tested	Dose Range and Solvent	Results* without S-9 Metabolic Activation					Results* with S-9 Metabolic Activation					Comments	Reference
		TA1535	TA1537	TA1538	TA98	TA100	TA1535	TA1537	TA1538	TA98	TA100		
2NPPD	10–50 μg/plate, dimethyl sulfoxide (DMSO)	–	–	(+)	–	–	–	–	(+)	–	–	–	74
2NPPD	50–100 μg/plate, DMSO	–	–	(+)	–	–	–	–	–	–	–	–	49
2NPPD	50 μg/plate, DMSO	(–)	–	–	(+)	(–)	(–)	–	–	(+)	(–)	–	76
2NPPD	12.5–100 μg/plate, DMSO	–	–	(+)	–	–	–	–	(+)	–	–	–	76
2NPPD	10–100 μg/plate, DMSO	–	–	(+)	(+)	(–)	–	–	(+)	(+)	(–)	–	78
2NPPD	10–666 μg/plate	–	–	–	(+)	–	–	–	–	(+)	–	–	79
2NPPD	–	–	(+)	(+)	–	(+)	–	(+)	(+)	–	(+)	–	79
2NPPD	50–100 μg/plate, DMSO	–	–	(+)	–	–	–	–	(+)	–	–	–	80
2NPPD	0.7 nmol/ml	–	–	(+)	(+)	(+)	–	–	–	–	–	–	84
2NPPD	50 μg/plate	–	(+)	(+)	–	–	–	–	(+)	–	–	–	97
2NPPD	15–150 μg/plate	–	–	–	(+)	–	–	–	–	(+)	–	2NPPD was mutagenic even after the addition of 5 percent hydrogen peroxide	88
4NOPD	10–50 μg/plate, DMSO	–	–	(+)	–	–	–	–	(+)	–	–	–	74
4NOPD	1–10 μg/plate, DMSO	–	–	(+)	–	–	–	–	–	–	–	–	49
4NOPD	50 μg/plate, water	–	–	–	(+)	–	–	–	–	–	–	–	75

4NOPD	0.1–1000 μg/ml, DMSO	(–)	(–)	(+)	(+)	(+)	(–)	(–)	(+)	(+)	(+)	4NOPD is also positive without S-9 in strain D3052 and is negative with and without S-9 in strains G46 and C3076	77
4NOPD	10–60 μg/plate, DMSO	–	–	(+)	(+)	(–)	–	–	–	–	–	–	78
4NOPD	0.3–333.3 μg/plate	–	–	–	(+)	–	–	–	–	(+)	–	–	79
4NOPD	–	–	–	(+)	(+)	–	(+)	–	–	–	–	–	79
4NOPD	50–100 μg/plate, DMSO	–	–	(+)	–	–	–	–	(+)	–	–	–	80
4NOPD	10 μg/plate, DMSO	–	–	–	(+)	–	–	–	–	–	–	–	81
4NOPD	50 μg/plate, DMSO	–	(+)	(+)	–	–	–	–	–	–	–	4NOPD is also positive without S-9 in strains TA97, TA90, and TA2637	82
4NOPD	–	–	–	(+)	–	–	–	–	–	–	–	4NOPD was mutagenic in TA1538 in plate tests and in liquid suspension tests only with greater than 20-hour incubations	83
4NOPD	33 nmol/ml	–	–	(+)	(+)	(+)	–	–	–	–	–	4NOPD was also positive without S-9 in strain D3052	84
4NOPD	5–50 μg/plate	–	(+)	(+)	–	–	–	–	(+)	–	–	–	97
4NOPD	10–40 μg/plate	–	(+)	(+)	(+)	(+)	–	–	–	–	–	–	86,87
4NOPD	15–150 μg/plate	–	–	–	(+)	–	–	–	–	(+)	–	4NOPD was mutagenic even after the addition of hydrogen peroxide	88
4NOPD	0.769–76.923 μg/ml	–	–	–	(+)	–	–	–	–	(+)	–	4NOPD was mutagenic even after the addition of hydrogen peroxide	89

*(+), mutagenic.
(–), nonmutagenic.

applied 4NOPD and the hair dyes were allowed to remain on the skin for 20 minutes, and then the rats were shampooed and rinsed. The urine from the rats receiving topical application of the two hair dyes, even when mixed with hydrogen peroxide prior to application, was positive for mutagenic activity. No mutagenic activity was detected in the urine of rats receiving topical applications of a hair dye not containing 2NPPD or 4NOPD.[49]

The urine of 30 women was evaluated for mutagenic activity. Fourteen of these women collected their urine before (first urine of the morning) and after (entire output for 24 hours) using hair dyes containing 0.007 to 0.09 percent 2NPPD and/or 0.007 to 0.154 percent 4NOPD. These 14 women were nonsmokers. (The other 16 women used hair dyes that did not contain 2NPPD and 4NOPD). The urine (in DMSO) was tested in the Ames test in *S. typhimurium* strain TA1538 with metabolic activation. The urine from these women was not more mutagenic after than before hair dye application.[99,100]

2NPPD and 4NOPD were "essentially unresponsive" in a mutagenesis assay with the tryptophan auxotrophs, *Escherichia coli* strains WP2 and WP2 uvrA,[101] and 4NOPD was active in an *E. coli* strain 343/113 arginine back mutation assay.[102] In a bacterial differential killing assay with *E. coli* strains WP2, WP67 uvrA polA, and CM871 uvrA recA lexA, 10 to 1000 μg/ml 4NOPD induced DNA damage.[103] Hair dyes containing 2NPPD and 4NOPD were negative in mutagenesis assays with *E. coli* strains WP2, WP2 uvrA, and WP2 exrA.[97,104]

4NOPD was positive in a *rec*-assay using *Bacillus subtilis.*[65] 4NOPD was also positive in a microsuspension modification of the *rec*-assay.[105] In 1976, MacGregor and Sacks tested 5 to 500 μg/ml 4NOPD in a multigene forward mutation test based on the sporulation system of *B. subtilis.* 4NOPD did not increase the mutation frequency of strain MC-1A, and little or no killing was found at the highest concentrations. The same researchers later reported that *B. subtilis* strain hcr-9 was sensitive to 50 to 500 μg/ml 4NOPD but that 4NOPD had no significant effect on *B. subtilis* strain 168.[106,107]

Yeast and Fungi

2NPPD and 4NOPD were not mutagenic using *Saccharomyces cerevisiae* strains D3 and D4 in plate test procedures with approximately 10 mg of chemical placed in the center of the plate. These compounds were also negative in 4- to 6-hour liquid suspension assays with 500 μg/ml of chemical with and without metabolic activation by mouse liver homogenate. In 48- to 96-hour liquid suspension assays without mouse liver homogenate in which yeast cells were treated with 500 μg/ml 2NPPD and 4NOPD under growing conditions, 2NPPD was negative and 4NOPD was positive.[108] 4NOPD, at concentrations of 20 to 100 μg/plate and 20 to 100 μg/ml, was not mutagenic to *S. cerevisiae* strain XV185-14C in plate and liquid suspension assays, respectively.[86,87]

2NPPD and 4NOPD were dissolved in DMSO, and 400 μg was placed on a disc on an agar plate. This dose of 2NPPD and 4NOPD was not mutagenic to *Neurospora crassa* strains N23 and N24.[109]

4NOPD was tested in a forward mutation assay with the fungus, *Aspergillus nidulans* haploid strain 35. It was not mutagenic in doses of 250 to 1000 μg/ml in a plate incorporation assay and in doses of 200 to 1200 μg/ml in a liquid-test assay. In both assays there was 100 percent survival of the fungus.[110]

L5178Y Mouse Lymphoma Cells

2NPPD and 4NOPD were assayed for mutagenic activity using the thymidine kinase locus of L5178Y mouse lymphoma cells.[111,112] The solvent for both chemicals was DMSO. 2NPPD was tested in concentrations of 25 to 75 μg/ml, and 4NOPD was tested in concentrations of 50 to 200 μg/ml. The assays were conducted without metabolic activation. Positive, dose-related responses were produced after a 24-hour exposure of the cells to 2NPPD and 4NOPD. Neither chemical was mutagenic after a 2-hour exposure of the cells.

The National Toxicology Program[113] reported that 2NPPD and 4NOPD were positive in the in vitro mouse lymphoma assay with and without metabolic activation.

Drosophila melanogaster

A 1.2 mM solution of 4NOPD in DMSO was fed to adult *Drosophila melanogaster* males for 3 days, and then they were mated. A 3-day brood was followed by two 2-day broods. Brood 1 represented mainly treated sperm, and broods 2 and 3 represented treated spermatids (and sperm) and spermatocytes (and spermatids), respectively. Sex-linked recessive lethal mutations were scored in the F_2 generation and were used as a measure of mutagenicity. 4NOPD was mutagenic with a peak activity in spermatids and spermatocytes, the metabolically active germ cells.[114]

D. melanogaster males were fed 0.003 percent 4NOPD in sucrose for 24 hours. 4NOPD did not induce *Minute* mutants in the F_1 generation or sex-linked recessive lethals in the F_2 generation.[115]

Fahmy and Fahmy[116] injected 5 to 20 mM 4NOPD in 2 percent (v/v) dimethylformamide around the testes of adult male *D. melanogaster* and examined the F_1 generation for *Minute* and rDNA mutations and the F_2 generation for sex-linked recessives (lethals and visibles). 4NOPD induced *Minute* mutants and exerted mutagenic effects on the RNA genes and the X-chromosome.

Sperm Abnormalities in Mice

4NOPD in water was administered intraperitoneally to groups of male mice for 5 consecutive days in doses of up to 2500 mg/kg per day. The mice were killed 35 days later, and their sperm were examined for abnormally shaped heads. 4NOPD was negative in the sperm abnormality assay.[75,117]

Chromosome Damage

Human Peripheral Blood Lymphocytes

Concentrations of 50 to 100 μg/ml 2NPPD caused chromosome and chromatid gaps and breaks in human peripheral blood lymphocytes cultures incubated for up to 72 hours. A concentration of 100 μg/ml 2NPPD resulted in mitotic delay and toxicity, and 45 percent of the cells contained damaged chromosomes. Chromosome damage after incubation with lower concentrations of 2NPPD or with 100 μg/ml 4NOPD was not significantly different from the controls.[97]

Chinese Hamster Cells

Chinese hamster cells were incubated for 24 hours with 1×10^{-5} to 2×10^{-4}

M 2NPPD and 1×10^{-5} to 3×10^{-4} M 4NOPD. Cells in metaphase were examined, and the number of chromatid breaks was increased after exposure to 2NPPD and 4NOPD. Several abnormal quadriradial and triradial, as well as dicentric chromosomes, were seen after incubation with 2NPPD.[118]

2NPPD and 4NOPD in DMSO caused chromatid gaps and breaks and translocations in Chinese hamster fibroblast cells in cultures incubated for 48 hours. The maximum effective dose of 2NPPD was 0.008 mg/ml (0.5×10^{-4} M), and the maximum effective dose of 4NOPD was 0.06 mg/ml (3.9×10^{-4}M).[119,120]

Kirkland and Venitt[121] found that 95-day continuous exposure of Chinese hamster prostate cells to 5 to 100 μg/ml 2NPPD and 4NOPD in DMSO was cytotoxic. Cells were exposed to 25 μg/ml 2NPPD and 4NOPD in DMSO, and the chromosomes were examined at times between 1 and 7 days after the start of the treatment. There was a time-dependent increase in chromosome aberrations. The observed damage included chromatid gaps and breaks, exchange figures, and dicentric chromosomes and other abnormal chromosomes.

Chinese hamster cells were scored for sister chromosome exchanges following a 24- to 34-hour incubation with 10 to 100 μg/ml 2NPPD in hot water or DMSO and 25 to 200 μg/ml 4NOPD in DMSO. All concentrations of both chemicals induced sister chromatid exchanges. High concentrations of 2NPPD and 4NOPD caused cell cycle delay.[122]

Micronucleus Test in Rats and Mice

Two doses of 2000 mg/kg 2NPPD and 5000 mg/kg 4NOPD in 0.5 percent (w/v) gum tragacanth given 24 hours apart were administered orally to groups of 5 male and 5 female rats. One animal given 4NOPD died. Both 2NPPD and 4NOPD resulted in the production of orange urine. Agitation, convulsions, and lethargy were observed in animals given either chemical. The rats were killed 6 hours after the second dose, and bone marrow smears were examined. Neither 2NPPD nor 4NOPD produced micronucleated polychromatic erythrocytes in rats.[123]

Groups of 2 male and 2 female mice were administered intraperitoneally two equal doses of 4NOPD in 3 percent gum arabic separated by a 24-hour interval. Doses of 4NOPD ranged from 75 to 300 mg/kg. The bone marrow of the mice was examined 6 hours after the second dose. 4NOPD did not produce micronucleated polychromatic erythrocytes in this study.[124] Other studies investigated the effect of the intraperitoneal administration for 5 consecutive days of doses of up to 2500 mg/kg/day 4NOPD in water on mouse bone marrow. The mice were killed 4 hours after the last injection. 4NOPD was negative in these mouse micronucleus assays.[75,117]

Dominant Lethal Assay in Rats

2NPPD and 4NOPD were administered intraperitoneally in a dose of 20 mg/kg three times a week for 8 weeks to groups of 20 male rats. Water was administered to a group of control rats. After the treatment period the male rats were mated with female rats for 5 days, and the female rats were killed 17 days later and their uteri examined. The numbers of live and dead fetuses and implantation and resorption sites in the females mated to treated males were not significantly different from the numbers in the females mated to control males. 2NPPD and 4NOPD did not increase postimplantation fetal loss, which would indicate a dominant lethal effect.[51]

Sheu and Green[67,68] administered intraperitoneally 10, 20, and 40 mg/kg 2NPPD and 25, 50, and 100 mg/kg 4NOPD in DMSO three times a week for 10 weeks to groups of 15 male rats. Control rats received DMSO. The highest dose reduced weight gains but was not lethal to most of the rats. After compound administration, each male rat was mated with 2 female rats each week for 2 weeks. The female rats were killed 17 days later and examined for live and dead implants. 2NPPD was negative and 4NOPD induced weak dominant lethality. The experiment was repeated with 4NOPD with groups of 20 rats, and the results were negative.

Aneuploid Production in Fungi

Exposure to 4NOPD did not increase the frequency of aneuploid products of meiosis in *N. crassa.*[125]

HeLa Cell DNA Synthesis Inhibition

4NOPD was negative with and without metabolic activation in the HeLa Cell DNA synthesis inhibition test. This assay tests for chemically caused DNA damage by measuring the inhibition of DNA synthesis after removal of the chemical from the medium.[126]

Unscheduled DNA Synthesis

HeLa Cells

The induction of unscheduled DNA synthesis in HeLa S3 cells was measured by autoradiography and used as a measure of DNA repair after exposure to 2NPPD and 4NOPD. Concentrations of 1×10^{-7} to 1×10^{-3} M 2NPPD and 1×10^{-3} M 4NOPD in DMSO induced unscheduled DNA synthesis in HeLa cells.[127]

Rat Hepatocytes

Unscheduled DNA synthesis was measured in primary cultures of adult rat hepatocytes after a 5-hour incubation with 0.5 to 1000 nmol/ml 2NPPD and 4NOPD in DMSO. Both chemicals were negative in the assay.[84,128] Other researchers performed the same experiment with an incubation of 18 to 20 hours. Concentrations of 1×10^{-1} to 1 mg/ml 2NPPD weakly induced DNA repair. 4NOPD at concentrations of 1×10^{-3} to 1×10^{-2} mg/ml did not induce unscheduled DNA synthesis.[129]

Morphological Transformation of Cells

Human Peripheral Blood Lymphocytes

Human peripheral blood lymphocytes are transformed in vitro to blastlike cells with the addition of phytohemaglutinin to cultures. Lymphocyte transformation was inhibited by a 48- to 72-hour incubation of the cells with 25 to 100 μg/ml 2NPPD and 4NOPD in water.[130]

C3H/10T 1/2 Mouse Cells

C3H/10T 1/2 CL8 mouse cells were examined for type III transformed foci 4 to 6 weeks after a 24-hour incubation with 2NPPD and 4NOPD in DMSO. Morphological transformation occurred after exposure to 1×10^{-5} to 1×10^{-3} M 2NPPD and 4NOPD. No type III foci were found after incubation of the cells with 1×10^{-6} M 4NOPD.[118]

Syrian Hamster Cells

Morphological transformation of cells occurred in Syrian hamster embryo cells incubated for 8 hours with 0.05 to 50 μg/ml 2NPPD and 4NOPD.[131-133]

Rat Embryo Cells

Rauscher leukemia virus-infected rat embryo cells were treated with 4NOPD (65 to 80 μg 4NOPD/5.2 × 10^4 cells) for 72 hours, and cell survival was determined 6 days later. This assay measures the acquisition of attachment independence, which is manifested by increased cell survival rates. A survival rate greater after treatment with chemical than after treatment with solvent alone would be a positive result. 4NOPD was positive at 80 μg/5.2 × 10^4 cells and negative at 65 μg/5.2 × 10^4 cells.[134]

Mouse Leukemia Virus Infection of Contact-Inhibited Cells

2NPPD and 4NOPD caused enhancement of infection of contact-inhibited C3H2K cells with Moloney mouse sarcoma-leukemia virus complex. The chemicals were dissolved in DMSO, serially diluted in ethanol, and incubated with the cells for 12 days. Maximum responses were seen with 10 μg/ml 2NPPD and 1 μg/ml 4NOPD.[135]

Carcinogenesis

Rats

2NPPD was fed in the feed for 78 weeks to rats, and the animals were observed for an additional 27 weeks. There were 20 control rats of each sex. Groups of 50 males were fed diets containing 550 and 1100 ppm 2NPPD, and groups of 50 females were fed diets containing 1100 and 2200 ppm 2NPPD. The rats were killed and necropsied at the end of the experiment. There were significant positive associations between dosage of 2NPPD and combined incidences of C-cell carcinomas or C-cell adenomas of the thyroid in male rats and between dosage of 2NPPD and combined incidences of leukemia and malignant lymphoma in female rats, but there were no significant Fisher exact comparisons to support these findings. The researchers concluded that "there was no convincing evidence for the carcinogenicity of . . . 2NPPD . . . in . . . rats."[25,55]

4NOPD, at dietary concentrations of 375 and 750 ppm, was fed to groups of 50 male and 50 female rats for 103 weeks, and the animals were observed for 2 additional weeks. There were 20 male and 20 female control rats. All the animals were killed and necropsied at the end of 105 weeks. There was no significant positive association between administration of 4NOPD and increased incidence of any tumor. It was concluded that 4NOPD, "under the conditions of this bioassay . . . was not carcinogenic in . . . rats."[26]

Mice

Groups of 50 male and 50 female mice were fed diets containing 2200 and 4400 ppm 2NPPD for 78 weeks and then were observed for an additional 12 weeks. There were 20 control mice of each sex. All the animals were killed and necropsied at the end of the experiment. The administration of 2NPPD was associated with a significantly increased combined incidence of hepatocellular ad-

enoma and hepatocellular carcinoma in female mice. These hepatocellular neoplasms occurred in a dose-related distribution. Tumor incidence was not statistically significant at any other site in female mice and at any site in male mice. The researchers concluded that 2NPPD "was carcinogenic to female . . . mice" and that "there was no convincing evidence for the carcinogenicity of . . . 2NPPD . . . in male . . . mice."[25,55]

Independent blind evaluations of slides of the mouse hepatic neoplasms by two pathologists (Paul M. Newberne, Ph.D., and Robert A. Squire, D.V.M., Ph.D.) resulted in a different conclusion. One pathologist found only one hepatocellular carcinoma, and it was in a low-dose male. The other neoplasms were hepatocellular adenomas of which most were very small and were considered benign neoplasms. In addition, he found increased foci of cellular alteration in high-dose females; these cells were similar in appearance to those in adenomas but were not considered neoplasms. He agreed that there was a treatment-related increase in adenomas in female mice. This pathologist concluded that the induction of only benign neoplasms indicated a proliferative stimulus that might be suggestive of a potential carcinogenic effect. A carcinogenic response was not clearly demonstrated. The other pathologist also stated that a carcinogenic effect was not demonstrated. He found an enhancement of parenchymal cell proliferation in treated female mice.[136]

4NOPD was administered in the feed for 102 weeks to mice, and the animals were observed for 2 additional weeks. There were 20 control mice of each sex. Groups of 50 males and 50 females were fed diets containing 3750 and 7500 ppm 4NOPD. All the mice were killed and necropsied at the end of 104 weeks. The incidence of hepatocellular adenomas was increased in treated female mice when compared to the controls, but the tumors were mostly in the low-dose group and their incidence was not statistically significantly different from the incidence in the controls. There was no significant positive association between administration of 4NOPD and increased incidence of any tumor. It was concluded that 4NOPD, "under the conditions of this bioassay . . . was not carcinogenic in . . . mice."[26]

A semipermanent hair dye was tested for carcinogenicity in strains A and DBAf mice by repeated topical application of the dyes. The dye contained 2NPPD and 4NOPD (in unspecified concentrations) and was used with a detergent in a shampoo base. It was diluted with 4 parts of water and 5 parts of acetone. A dose of 0.4 ml of the hair dye and the detergent in a shampoo base was applied to the clipped backs of strain A mice. The same dose was applied to the DBAf mice for 24 weeks and then the dose was reduced to 0.2 ml due to the observation of toxic effects in the urogenital tract. The dye was usually applied twice weekly. The mice received a total of 138 applications over 80 weeks. The major findings are summarized in Table 6 and show "that the treatments of the strain A mice resulted mainly in small acceleration of the appearance of spontaneous lymphoid tumors. In DBAf mice, however, there was both an earlier appearance and an increased incidence of tumors. The excess was due mainly to uterine, ovarian and skin tumors which were not seen in the control group." Statistically significant excess of lymphomas and other tumors could not be demonstrated, possibly because of the small number of mice per group.[62,85,97,98]

Hair dye composite formulations containing 1.1 percent 2NPPD or 0.25 percent 4NOPD were mixed 1:1 with 6 percent hydrogen peroxide, and 0.05 ml of

TABLE 6. Incidence of Tumors in A and DBAf Mice Treated by Repeated Applications of a Semipermanent Hair Dye

	Mice Treated			Examined at	Mice with Tumors			
Strain	*Treatment*	*Sex*	*No.*	*Postmortem*	*Lymphomas (weeks)*	*Other Tumors*	*Weeks*	*Mice Tumor-Free (weeks)**
A	Control	M	16	16	77 80	Hepatoma	80	60 75 80 (8)
						Lung adenoma	80 80 80	
		F	16	16	61 75 80 80 80	Lung adenoma	75 80	36 50 59 72 80 (5)
	Dye	M	26	25	57† 57 80† 80	Hepatoma	80†	33 49 56 69 69 71 75 77 80 (9)
						Lung adenoma	57† 79 80 80 80	
		F	26	23	48 65 80 80	Lung adenoma	51 78 80 80	46 55 66 72 78 80 (10)
DBAf	Control	M	16	15		Hepatoma	80	48 65 70 71 80 (10)
		F	16	15	72	Lung adenoma	80	48 61 80 (11)
	Dye	M	26	23	26	Penile skin papilloma	29 39 47	22 31 32 48 51 53 58 61 63 66 70 73 76 77 79 80 (3)
		F	22	18	37 41 73 80†	Ovarian cystadenoma	80† 80 80 80	59 73 78 79 80 (5)
						Uterine fibrosarcoma	66 69	

*No. of tumor-free mice at 80 weeks in parentheses.
†Mouse with additional primary tumor.

the mixture was applied topically to the clipped intrascapular areas of groups of 50 male and 50 female mice once weekly for 21 to 23 months. At 7 and 9 months, 10 male and 10 female mice from each group were killed and necropsied. Gross and microscopic examinations were made of organs of all the mice that died and those killed at the termination of the experiment. There were three shaved but untreated control groups. The incidences of tumors in controls and treated groups were not significantly different. Carcinogenic effects were not induced by the hair dye formulations.[63]

Two hair dye composite formulations containing 1.1 percent 2NPPD or 0.25 percent 4NOPD were applied topically to rats (the F_0 generation) from the time of weaning to the weaning of their young (the F_{1A} generation). The hair dyes were mixed 1:1 with 6 percent hydrogen peroxide and applied topically two times a week for 2 years to the clipped backs and necks of groups of 60 male and 60 female rats of the F_{1A} generation. The rats received an initial application of 0.2 ml and this was increased by 0.1 ml weekly to 0.5 ml. Ten rats from each group were killed and necropsied at 12 months, and all other rats were necropsied at their deaths or at experiment termination. There were three clipped but untreated control groups. There were no compound-related gross lesions observed in any of the rats. The stratum corneum of the skin and of the hair shafts of the treated rats was colored by the dye. Various tumor or tumorlike lesions were observed in all the groups in low incidences.[64]

CLINICAL ASSESSMENT OF SAFETY

Patch tests were performed with 1 percent PPDA on 2094 subjects in the United States in 1979 and 1980. Six percent (136 subjects) had positive reactions for PPDA.[137]

Thirty-nine hairdressers were patch tested for 24 hours with 2NPPD. Thirty-two of the hairdressers had no history of allergic contact dermatitis from *p*-phenylenediamine, and none of these 32 had a positive reaction to 2NPPD. One of seven hairdressers who had experienced strongly positive reactions to *p*-phenylenediamine was positive for 2NPPD. The researchers suggested that this may have been a cross reaction.[65]

A repeated insult patch test was conducted with a hair dye containing 0.027 percent 4NOPD and 0.49 percent PPDA.[138] Two hundred six subjects were enrolled in and completed the study. The dye was mixed with an equal volume of oxidizer, and each nonocclusive patch contained 0.1 ml per cm^2 of the dye and oxidizer mixture. Ten 48- to 72-hour consecutive patch applications were made on the backs of the subjects, and reactions were read after removal of each patch. These induction patches were followed by an 11-day rest period. A 48-hour nonocclusive challenge patch was applied to a previously unexposed site on the back of each subject, and the reaction was read at removal and at 15 minutes and at 24 hours later. There were no positive reactions at any induction or challenge reading. The researchers stated that their data provided no evidence to indicate that the hair dye and oxidizer test product caused either irritation or sensitization. A repeated insult patch test was conducted with a hair dye containing 0.039 percent 4NOPD and 0.4 percent PPDA on the same 206 subjects and following the same procedure.[139] There were 41 doubtful reactions (very mild

erythema, barely exceeded that of untreated skin) during induction. There were no positive reactions at any induction or challenge reading. The researchers stated that their data provided no evidence to indicate that the hair dye and oxidizer test product caused either irritation or sensitization. A repeated insult patch test was conducted with a hair dye containing 0.049 percent 4NOPD and 0.596 percent PPDA on the same 206 subjects and following the same procedure.[140] There were no positive reactions at any induction or challenge reading. The researchers stated that their data provided no evidence to indicate that the hair dye and oxidizer test product caused either irritation or sensitization.

A case has been described in the literature in which a dental hygienist developed dermatitis on the skin of her left forearm where it came into frequent contact with the hair of patients. A patch test with *p*-phenylenediamine mix was negative at 48 and 96 hours, and, with 2 percent 2NPPD, was strongly positive at 48 hours.[141]

EPIDEMIOLOGY

A variety of published studies have assessed the association between occupational exposure to, and use of, hair dyes and the risk of cancer. These studies do not distinguish which of specific hair dye ingredients were involved in the human exposure. The reader is referred to the literature for the specific results and interpretations of the investigators. A summary of reports of how occupational exposure to hair dyes affects the risk of bladder cancer[142–145] and lung cancer,[146,147] or the use of hair dyes affects the risk of bladder cancer in men or women[148] and breast cancer in women,[149–154] can be found in Table 7.

In a 1979 Federal Register,[155] the FDA stated that existing epidemiological evidence did not indicate hair dyes caused human cancer. Clemmesen[156] discussed the difficulties implicit in epidemiological studies and reviewed many of the papers that investigated the relationship of the risk of cancer to occupational exposure to, or use of, hair dyes. He concluded that most researchers used samples that were too small to allow conclusions and analyses of duration and intensity of exposure, lag time, and the influence of lifestyle factors, such as tobacco use, were deficient in many cases. Clemmesen[156] stated that there was no evidence of any carcinogenic effect from hair dyes on the organs investigated among the occupations and users examined.

SUMMARY

2NPPD and 4NOPD are substituted aromatic amines used in semipermanent and permanent hair dye formulations. In 1981, it was reported to the FDA that 2NPPD and 4NOPD were used in concentrations ranging from ≤ 0.1 percent to 1 percent in totals of 28 and 26 hair dyes and colors, respectively, and 4NOPD was used in concentrations of ≤ 0.1 percent in 6 hair tints. Hair dyes containing 2NPPD and 4NOPD are exempt from the color additive provisions in sections 601 and 706 of the Federal Food, Drug and Cosmetic Act of 1938 when their labels bear a conspicuously displayed caution statement and patch test instructions for determining whether the product causes skin irritation.

2NPPD (^{14}C) was administered intraperitoneally or intravenously to rats. Ra-

dioactivity was distributed throughout the body, and the greatest percentages were found in the muscle, large and small intestines, and liver. Radioactivity was excreted in the bile, feces, and urine. Nonmetabolized 2NPPD, N^4-acetyl-2-nitro-*p*-phenylenediamine, and N^1,N^4-diacetyl-2-amino-*p*-phenylenediamine, and two unidentified metabolites were detected in the urine. 2NPPD (^{14}C) was applied topically to rhesus monkeys and white swine, and radioactive label was excreted in the urine. Rats were intubated or injected intraperitoneally with 2NPPD (^{14}C). Radioactivity was recovered from the urine and feces but not from expired air. Unchanged 2NPPD, acetylated 2NPPD, sulfate and/or glucuronide conjugates of 2NPPD and of acetylated 2NPPD, and two conjugates with sulfur-containing amino acids were detected in the feces. 2NPPD (^{14}C) was applied topically to rats; it was absorbed and excreted in the urine and feces. The same pattern of radioactivity among metabolites was observed as in the orally or parenterally dosed animals. 2NPPD (^{14}C) in a hair colorant base was applied topically to rats; it was absorbed and excreted. Skin penetration varied with treatment after application (rinsed or not, occlusive or nonocclusive patch), length of application, number of applications, concentration of 2NPPD in the base, and whether the application site was clipped or left hairy. A semipermanent hair dye and base composite containing 2NPPD and 4NOPD was administered orally to rabbits and given in feed to dogs and rats; the animals excreted blue-brown urine. 4NOPD and a hair dye containing 4NOPD were injected intraperitonealy into rats; the urine of the rats was mutagenic for *S. typhimurium*. 4NOPD and hair dyes containing 2NPPD and 4NOPD were also applied topically to rats; the urine from these rats was mutagenic using *S. typhimurium* strains.

The acute oral LD_{50} for rats for 2NPPD ranged for 1800 to 3080 mg/kg, and the LD_{50} for rats for 4NOPD ranged from 681 to 3720 mg/kg; 2NPPD and 4NOPD were slightly toxic. Mice were fed up to 11,830 ppm 2NPPD in their diet for 4 weeks or up to 4400 ppm for 78 weeks, and rats were fed up to 6800 ppm for 4 weeks or 1100 ppm for 78 weeks; no adverse effects were observed. Mice were fed up to 21,500 ppm 4NOPD in their diet for 7 weeks and orange-colored fur was observed. Dietary concentrations of up to 7500 ppm for 102 weeks resulted in a dose-related mean weight depression. Rats were fed 10,000 ppm 4NOPD in their diet for 7 weeks, and arched backs and rough coats were observed. No signs of toxicity were observed when rats were fed concentrations of up to 750 ppm 4NOPD for 103 weeks. There were no adverse effects when a semipermanent hair-coloring composite containing 0.24 percent 2NPPD and 0.16 percent 4NOPD was fed at a concentration of 7800 ppm for 9 days to pregnant rats or for more than 8 weeks to nonpregnant rats. A 97.5 mg/kg per day oral dose of this composite was administered to pregnant rabbits for 12 days and to dogs for 2 years; no signs of toxicity were observed.

No deaths occurred in rabbits after the topical application of 5 g/kg of 2NPPD and 4NOPD in a hair dye base. Hair dye composites containing 0.55 percent 2NPPD and 0.013 and 0.036 percent 4NOPD were applied topically to rabbits daily for 20 days. There were no signs of toxicity and no significant gross abnormalities at necropsy 14 days after the test period. There were local skin reactions during the test period, but the hair and skin appeared normal by the end of the 14-day observation period following the test period. Hair dye composites containing 0.55 and 1.1 percent 2NPPD and 0.25 percent 4NOPD were applied to the hair and skin of rabbits once every 2 weeks or twice a week for 13 weeks

TABLE 7. A Brief Summary of Reports on Cancers Associated with Exposure to Hair Dyes

Population Studied	*Comments*	*Reference*
	Occupational Exposure to Hair Dyes	
1030 bladder papilloma and carcinoma patients were interviewed for occupational history in Leeds, England, from 1959 to 1967. 383 male and 57 female bladder tumor patients were matched for sex, age decade, habitat, and smoking habits with 340 male and 50 female surgical controls and 312 male and 39 female patients with cancer at other sites	There were consistently nonsignificant differences found for male hairdressers (predominant occupation). There were 4, 1, and 0 hairdressers among 383 bladder tumor patients, 340 surgical controls, and 312 cancer controls, respectively. Men employed as hairdressers for less than 20 years were less likely to have bladder tumors than those employed for longer than 20 years; male hairdressers with bladder tumors had lower mean ages at diagnosis compared to the whole interviewed series. The population of males with bladder tumors contained more hairdressers than expected; 5 were observed, 1.8 and 1.5 were expected in 1961 and 1951, respectively (based on census data)	142
461 persons of ages 20–89 with transitional or squamous-cell carcinoma of the lower urinary tract (94 percent had a bladder tumor) interviewed for occupational history in an 18-month period in an area of eastern MA. 356 male and 105 female persons with a bladder cancer were matched for sex, age, and/or smoking with 374 male and 111 female controls	Cigarette smoking was not responsible for an indirect association of bladder cancer risk and occupation. Of the persons with bladder cancer, 4 were male barbers and 1 was a female hairdresser. 7.2 and 0.9 were expected, respectively. The researchers stated that the data do not support a suggestion of increased bladder risk for barbers, but that the number of observations was too low and therefore, inadequate to exclude the possibility of increased risk. No excess risk was found for female hairdressers	143
702 patients with presumptive or confirmed diagnosis of bladder tumors. 493 bladder cancer patients (265 male whites, 69 male blacks, 112 female whites, and 47 female blacks) and 527 patient controls were interviewed for occupational history from 1958 to 1964 in New Orleans, LA	There was no clear correlation between bladder cancer and occupation or industry. For male whites with bladder cancer, 4 were barbers at the time or had been barbers as a final occupation. 1.45 were expected. The researchers had doubts about the validity of their analytical method and did not conclude that being a barber increased risk to bladder cancer. Further interviews with 7 male barbers and 2 female hairdressers with bladder cancer indicated wide differences in their occupation, starting ages, years in occupation, and age at diagnosis of bladder cancer	144
300 male and 70 female bladder cancer patients from 1957 to 1961 in New York City were matched by sex and age with the same number of control patients. All the subjects were interviewed about their occupations	There were 4 hairdressers in the male bladder cancer group, 3 of whom had been hairdressers for more than 5 years. There were no hairdressers in the male control group. There was one beautician in the female bladder cancer group. The researchers drew no definite conclusions	145

The death certificates of 3460 adult (≥ 14 years of age) females who died of cancer and 1000 females who died from some other cause in Alameda County, CA from 1958 to 1962 were examined. Cancer cases and controls were matched for age and sex	24 of the 3460 females who died of cancer and 4 of the 1000 controls were beauticians. The risk of cancer death for beauticians was elevated but not significantly. Six of the 24 beauticians who died of cancer and 170 of the 3436 females of other occupations who died of cancer had lung cancer. The small numbers inject uncertainty, but the researchers suggested that the risk of lung cancer may be substantially increased among beauticians	146
Examined hospital records from Los Angeles County for 1972 to 1975. 22792 white women with cancer, 20–64 years old, were admitted and 9524 of the women reported occupations	Of the 22792 women, 135 were beauticians, and 20 of the beauticians had lung cancer. 32, 22, and 15 percent of the beauticians had breast, genital, and lung cancer, respectively. Only the lung cancer incidence was significant compared to the expected frequencies for age and sex calculated from the census data	147
	Use of Hair Dyes	
107 bladder cancer patients and 107 controls were matched by age (± 5 years) and sex. Male controls were patients with benign prostatic hypertrophy, female controls had been seen with problems of stress incontinence (Toronto, Ontario, Canada)	No statistically significant difference was found between cancer and control groups in reported exposure to hair dyes	148
Surveyed 120,557 married, female, registered US nurses, from 10 states. 38,459 (31.9 percent) had used permanent hair dyes and 3548 (2.9 percent) had had cancer	Statistically significant associations with hair dye use were found only for cancers of the cervix uteri and vagina and vulva. Women who had used hair dye ≥ 21 years prior to diagnosis of breast cancer had significantly greater risk for all sites – mostly due to the excess number of observed to expected cases of breast cancer. However, those who used hair dyes 16 to 20 years prior to diagnosis of breast cancer had an almost equal deficit of observed to expected breast cancers. Adjustments for smoking did not change the results. The researchers concluded that there was no evidence of increased risk of cancer during the initial 20 years	149
191 female breast cancer patients matched for age (within 3 years), marital status, and social class with 561 inpatient, outpatient, or general practice controls (Oxford, England, 1975–1976)	There were no significant differences in the use of hair dyes by breast cancer patients and controls. The frequency of applications and brands used by hair dye users in both groups were approximately the same. There were no significant differences when the analysis was restricted to women who had used hair dyes >4 or >9 years prior to breast cancer diagnosis.	150
118 breast cancer patients of ages 20 to 84 (from 3 upstate New York counties). 233 controls selected by "random digit dialing" of the telephone. Cancer patients and controls matched by age and county	No significant differences observed between breast cancer patients and controls in exposure to hair dyes. Hair dye use was marginally significantly associated with breast cancer in women 40–49 years old. Previous benign breast disease and hair dye exposure significantly	151

TABLE 7. (Continued)

Population Studied	*Population Studied*	*Comments*	*Reference*
		increased a woman's risk of developing breast cancer. A significant dose–response relationship between number of hair dye exposures and breast cancer was observed for women who did not have gray hair and used hair dyes to change their natural hair color	
Reviewed case histories of 100 breast cancer patients. Compared these to a study of women of the same age who did not have breast cancer (New York)		87/100 of the breast cancer patients were regular users of permanent hair coloring and had used hair dyes for more than 5 years. 26 percent of the women without breast cancer were regular users of permanent hair dyes over prolonged periods	152
129 breast cancer patients and 193 female controls without breast cancer selected from a breast cancer screening center in New York City from 1964 to 1976		There were no significant differences between the cancer patients and controls in use of hair dyes prior to breast cancer diagnosis. However, there was a difference in the integral (frequency × duration) use of dyes for the 2 groups. The association between integral use and breast cancer was clearest when hair dye was used for at least 10 years prior to cancer diagnosis. The association of integral use and breast cancer occurred primarily among women 50 to 79 years old	153
401 breast cancer patients and 625 age-matched controls without breast cancer from a cancer referral center in New York City from 1979 to 1981		There were no significant differences between the breast cancer patients and controls with regard to hair dye use: frequency, duration, type, shade, or application time. Important confounders of hair dye use included religion and smoking status	154

and allowed to remain on the skin for 1/2 to 1 hour; there were no adverse findings. Topical application of hair dyes containing 1.1 percent 2NPPD and 0.25 percent 4NOPD every third day during the gestation of rats did not result in any signs of toxicity. A semipermanent colorant shampoo containing 2NPPD and 4NOPD (unspecified concentrations) was applied to the skin of two mouse strains two times a week for 80 weeks. Toxic effects were seen in one mouse strain; obstructing crystals were seen in the urinary bladder and on the penile skin. The urinary bladder, seminal vesicles, and stomachs were distended, the renal tubules were dilated, and a few mice had chronic gastritis. Hair dye composites containing 1.1 percent 2NPPD and 0.25 percent 4NOPD were applied topically to mice for 21 to 23 months; there were no adverse findings. The same hair dye composites were applied topically in a two-generation rat study; no signs of toxicity were observed.

The primary skin irritation of 2NPPD and 4NOPD has been determined in rabbits. Concentrations of 2.5 percent 2NPPD and 4NOPD were not irritating (PII was 0 for both compounds), and 5 percent 4NOPD was only slightly irritating (PII was 0.38). Three percent 2NPPD and 4NOPD inductions, followed by a rest period and a challenge patch, resulted in the sensitization of 4 of 20 and 18 of 20 guinea pigs, respectively. One in ten guinea pigs previously sensitized to *p*-phenylenediamine was also sensitive to 4NOPD.

Concentrations of 2.5 percent 2NPPD and 4NOPD and 5 percent 4NOPD (score was 3.0 of possible total of 110) were only slightly irritating to the rabbit eye.

The acute intraperitoneal LD_{50}s of 2NPPD and 4NOPD were 348 mg/kg and greater than 1600 mg/kg, respectively. The intraperitoneal administration to rats of 40 mg/kg per day 2NPPD and 100 mg/kg per day 4NOPD for 5 days, or 20 mg/kg 2NPPD and 4NOPD three times a week for 8 weeks, suppressed body weight gains. Doses of 2NPPD of up to greater than 160 mg/kg per day and 4NOPD of up to greater than 256 mg/kg per day to pregnant mice for 9 days during gestation resulted in a significant decrease in maternal weight gain; significant maternal toxicity was observed at doses of 160 mg/kg per day or more of 2NPPD.

Subcutaneous administration of doses of 2NPPD equal to or greater than 160 mg/kg per day and of 4NOPD equal to or greater than 256 mg/kg per day to pregnant mice significantly increased the number of fetuses with cleft palates and blood vessel malformations. Average fetal weight was decreased at this dose of 4NOPD and at 125 mg/kg per day 2NPPD or greater. Dose-related increases in resorption incidence and number of stunted fetuses was observed with 2NPPD. Two hair dyes containing 1.1 percent 2NPPD and 0.25 percent 4NOPD were applied topically in a dose of 2 ml/kg to pregnant rats; the hair dyes had no embryotoxic or teratogenic effects. Hair dyes containing the same concentrations of 2NPPD (1.1 percent) and 4NOPD (0.25 percent) were applied topically in doses of 0.5 ml two times a week to mice for three generations. Local skin reactions were noted, but no other adverse effects were observed. A hair dye formulation containing 1.1 percent 2NPPD and 0.25 percent 4NOPD was applied topically in a dose of 0.05 ml two times a week to female mice prior to mating and throughout mating and gestation. There was no evidence of a teratogenic effect, but there may have been a retarding effect on the ossification process, particularly of the bones of the feet and of the crevical and caudal vertebral centra. The same hair dye formulation (1.1 percent 2NPPD and 0.25 percent 4NOPD) was applied

topically in a dose of 2 ml/kg to female rabbits prior to mating and throughout mating and gestation. There was no evidence of a teratogenic effect. There may have been some evidence of embryotoxicity; the percent of live fetuses was less and the percent of resorbed fetuses was greater in the treated rabbits than in the control rabbits. A semipermanent hair-coloring composite containing 0.24 percent 2NPPD and 0.16 percent 4NOPD was administered in concentrations of up to 7800 ppm in the feed of pregnant rats or to male or female rats prior to mating, during mating, and throughout gestation and lactation. The composite was not embryotoxic or teratogenic. The same hair-coloring composite was administered orally in doses up to 97.5 mg/kg to pregnant rabbits. Embryotoxicity and teratogenicity were not observed.

2NPPD and 4NOPD were mutagenic for some strains of *S. typhimurium* with and without metabolic activation. 4NOPD is used as a positive control for *S. typhimurium* strains TA1538 and TA98 without metabolic activation and can be used in a scheme to confirm the phenotype of the standard tester strains. Several hair dyes containing 2NPPD and 4NOPD were mutagenic for some *S. typhimurium* strains. Mutagenic activity toward *S. typhimurium* was detected in the urine of rats after the intraperitoneal or topical application of 4NOPD or hair dyes containing 2NPPD and 4NOPD; mutagenic activity was not detected in the urine after the topical application of hair dyes not containing 2NPPD or 4NOPD. The urine of women who used hair dyes containing 2NPPD and/or 4NOPD was not more mutagenic in the Ames test than their urine prior to hair dye use.

2NPPD and 4NOPD were not mutagenic in an assay using two strains of *E. coli*, and 4NOPD was mutagenic in an assay using another *E. coli* strain. 4NOPD induced DNA damage in several *E. coli* strains. Hair dyes containing 2NPPD and 4NOPD were negative in mutagenesis assays with several strains of *E. coli*. 4NOPD was positive in *rec*-assays with *B. subtilis*. Depending on the strain, 4NOPD was positive or negative in a *B. subtilis* multigene forward mutation test. 2NPPD was not mutagenic to the yeast, *S. cerevisiae*, in plate and liquid suspension assays. Results were negative for 4NOPD in plate assays and were positive or negative in liquid suspension assays depending on the strain of the yeast and the length of the assay. 2NPPD and 4NOPD were not mutagenic to the fungus, *N. crassa*, and 4NOPD were not mutagenic to the fungus, *A. nidulans*.

2NPPD and 4NOPD were reported by two research groups to be mutagenic in L5178Y mouse lymphoma cells. 4NOPD was fed (1.2 mM solution) to *D. melanogaster* males, and sex-linked recessive lethal mutations were observed in the F_2 generation. In another study, feeding 4NOPD (0.003 percent in sucrose) to flies did not induce *Minute* mutants in the F_1 generation or sex-linked recessive lethals in the F_2 generations. Other researchers injected 4NOPD (5 to 20 mM) around the testes of male *D. melanogaster* and determined that *Minute* and rDNA mutations were induced in the F_1 generation and sex-linked recessives were induced in the F_2 generation. 4NOPD was negative in the mouse sperm abnormality assay.

2NPPD caused chromosome damage in human peripheral blood lymphocytes; 4NOPD was not active. Both compounds caused chromosome damage in Chinese hamster cells; damage included sister chromatid exchanges. 2NPPD and 4NOPD were negative in a rat micronucleus test and 4NOPD was negative in a mouse micronucleus test. 2NPPD and 4NOPD were negative in dominant lethal assays in rats. 4NOPD did not increase the frequency of aneuploid products of meiosis in the fungus, *N. crassa*.

4NOPD did not damage HeLa cell DNA in one study; DNA synthesis after removal of 4NOPD from the cell medium was not inhibited. In another study, HeLa cell DNA damage was measured by observing unscheduled DNA synthesis; both 2NPPD and 4NOPD induced unscheduled DNA synthesis in HeLa cells. These compounds did not induce unscheduled DNA synthesis in rat hepatocytes.

Human peripheral blood lymphocytes are transformed in vitro to blastlike cells with the addition of phytohemaglutinin to cultures; 2NPPD and 4NOPD inhibited this transformation. C3H/10T CL8 mouse cells and Syrian hamster embryo cells were transformed after exposure to 2NPPD and 4NOPD. 4NOPD was positive in a virus-infected rat embryo cell survival assay.

2NPPD and 4NOPD caused enhancement of infection of contact-inhibited C3H2K cells with mouse leukemia virus.

Male rats and female rats were fed diets containing up to 1100 ppm 2NPPD and 2200 ppm 2NPPD, respectively, for 78 weeks. Although there were significant positive associations between 2NPPD dosage and some types of cancer, there was no convincing evidence for carcinogenicity of 2NPPD in rats. Concentrations of up to 750 ppm 4NOPD were administered in the feed to rats for 103 weeks. There was no significant positive association between 4NOPD administration and increased incidence of any tumor. 4NOPD was not carcinogenic to rats.

Mice were fed diets containing up to 4400 ppm 2NPPD for 78 weeks. The researchers reported the administration of 2NPPD was associated with a significant dose-related increase in the combined incidence of hepatocellular adenoma and hepatocellular carcinoma in female mice, and concluded that 2NPPD was carcinogenic to female mice. Two pathologists performed evaluations of the slides of the mouse hepatic tumors and stated that a carcinogenic effect was not demonstrated in the study. Concentrations of up to 7500 ppm 4NOPD were administered in the feed to mice for 102 weeks. There was no significant positive association between administration of 4NOPD and increased incidence of any tumors. 4NOPD was not carcinogenic to mice.

A semipermanent hair dye containing unspecified concentrations of 2NPPD and 4NOPD was applied topically to two strains of mice approximately two times a week for a total of 138 applications over 80 weeks. In one strain of treated mice there was an earlier appearance and greater incidence of uterine, ovarian, and skin tumors than in the controls; the researchers reported that the dye appeared to be carcinogenic to the one strain of mice. Carcinogenic effects were not induced in mice after the weekly topical application for up to 23 months of hair dye composites containing 1.1 percent 2NPPD and 0.25 percent 4NOPD. Two hair dye composites containing the same concentrations of 2NPPD (1.1 percent) and 4NOPD (0.25 percent) were applied topically to rats (the F_0 generation) from the time of weaning to the weaning of their young (the F_{1A} generation). The composites were applied topically two times a week for 2 years to the F_{1A} rats. There were no compound-related gross lesions observed in any of the rats.

Thirty-nine hairdressers were patch-tested with 2NPPD. Seven had previously had strong reactions to *p*-phenylenediamine. One of the seven was positive for 2NPPD. The other 38 hairdressers did not react to 2NPPD. Repeated insult patch tests were conducted on 206 volunteers with three hair dyes containing up to 0.049 percent 4NOPD and 0.596 percent PPDA. There were no positive reactions at any induction or challenge reading; the hair dye was not an irritant or

sensitizer. A dental hygienist who had developed dermatitis where her skin came in contact with the hair of patients was patch-tested with *p*-phenylenediamine and 2NPPD; she had a positive reaction only for 2NPPD.

A variety of epidemiological studies assess whether and to what degree occupational exposure to, and use of, hair dyes increases the risk of cancer. The results of these studies vary widely. The Food and Drug Administration has stated that existing epidemiological evidence does not indicate that hair dyes cause cancer in humans. A review of many of the epidemiological studies concludes that there was no evidence of a carcinogenic effect from hair dyes on the organs investigated among the occupations and users examined.

DISCUSSION

This report on 2NPPD and 4NOPD reviews data obtained from animal studies, but little clinical data were available for review. The animal data suggest that both compounds are nonirritating to rabbit skin and eyes and are sensitizers for guinea pig skin. In repeated insult patch tests with hair dyes containing 4NOPD and PPDA, the hair dyes were not irritants or sensitizers. In the absence of human data on the pure compound, 2NPPD and 4NOPD should be considered to have high potential for human sensitization.

2NPPD and 4NOPD are frequently used in hair dye formulations with PPDA. Therefore, toxicity data for PPDA may be relevant for the evaluation of the toxicity of hair dyes containing 2NPPD and 4NOPD. Patch tests with 1 percent PPDA (2049 subjects) resulted in 6 percent (136 subjects) positive reactions.

2NPPD and 4NOPD are mutagenic in some bacterial and in vitro mammalian systems; both compounds have some genotoxic activity. In feeding studies in mice and rats, only 2NPPD induced hepatocellular tumors in female mice. Both compounds were noncarcinogenic in male mice and in rats. Epidemiological data have not demonstrated a carcinogenic effect in man (on the bladder, lung, or breast) for hair dyes.

CONCLUSION

2NPPD and 4NOPD are skin sensitizers for guinea pigs. Information in this report and in the report on PPDA suggests that 2NPPD and 4NOPD have potential for human sensitization. For those persons not sensitized, the Expert Panel concludes that 2NPPD and 4NOPD are safe as hair dye ingredients at the current concentration of use.

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REFERENCES

1. ESTRIN, N.F., CROSLEY, P.A., and HAYNES, C.R. (eds.). (1982). *CTFA Cosmetic Ingredient Dictionary*, 3rd ed. Washington, DC: Cosmetic Toiletry and Fragrance Association.
2. INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (IARC). (1978). IARC *Monographs on the Car-*

cinogenic Risk of Chemicals to Man. Some aromatic amines and related nitro compounds – Hair dyes, colouring agents and miscellaneous industrial chemicals. Lyon, France: IARC, Vol. 16.
3. WINDHOLZ, M. (ed.). (1976). *The Merck Index*, 9th ed. Rahway, NJ: Merck & Co.
4. COSMETIC, TOILETRY AND FRAGRANCE ASSOCIATION (CTFA). (November 16, 1982). Cosmetic ingredient chemical description, 2-nitro-*p*-phenylenediamine.*
5. GREENBURG, L.A., and LESTER, D. (1954). *Handbook of Cosmetic Materials.* New York: Interscience Publishers.
6. THE SOCIETY OF DYERS AND COLOURISTS. (1971). *Colour Index*, 3rd ed. Yorkshire, UK, Vol. 4.
7. CTFA. (November 16, 1982). Cosmetic ingredient chemical description, 4-nitro-*o*-phenylenediamine.*
8. WEAST, R.C. (ed.). (1978). *CRC Handbook of Chemistry and Physics*, 5th ed. West Palm Beach, FL: CRC Press.
9. REIO, L. (1970). Third supplement for the paper chromatographic separation and identification of phenol derivatives and related compounds of biochemical interest using a "reference system." J. Chromatogr. **47,** 60–85.
10. TURCHETTO, L., CUOZZO, V., TERRACCIANO, M., PAPETTI, P., and PERCACCIO, G. (1978). Analytical study of certain hair dyes. Boll. Chim. Farm. **117,** 475–8.
11. MIDLER, O., and KARLESKIND, A. (1978). Hair dyes acting by oxidation. Their identification and estimation by high-performance liquid phase chromatography. Parfums. Cosmet. Aromes **23,** 77–80, 83–5.
12. GOLDSTEIN, S., KOPF, A.A., and FEINLAND, R. (April 21–23, 1968). Analysis of oxidation dyes in hair colorants by thin-layer and gas chromatography. Proc. Joint Conf. Cosmet. Sci. Washington, DC: Toilet Goods Assoc.
13. KOTTEMANN, C.M. (1966). Two-dimensional thin-layer chromatographic procedure for the identification of dye intermediates in arylamine oxidation hair dyes. J. Assoc. Off. Anal. Chem. **49,** 954–9.
14. LEPRI, L., DESIDERI, P.G., and COAS, V. (1974). Chromatographic and electrophoretic behavior of primary aromatic amines on anion-exchange thin layers. J. Chromatog. **90,** 331–9.
15. LEPRI, L., DESIDERI, P.G., and COAS, V. (1976). Separation and identification of coloring agents in the oxidation-type hair dyes by ion-exchange thin-layer chromatography. Ann. Chim. (Rome) **66,** 451–600.
16. PINTER, I., and KRAMER, M. (1966). Le dosage de quelques diamines aromatiques employees dans la teinture pour cheveux par chromatographie sur couche mince. Arch. Biochim. Cosmetol. **9,** 153–7.
17. PINTER, I., and KRAMER, M. (1967). The determination of some aromatic diamines used in hair dyes by thin-layer chromatography. Parfum. Cosmet. Savons **10,** 257–60.
18. SENZEL, A.J. (1977). *Newburger's Manual of Cosmetic Analysis*, 2nd ed. Washington, DC: Assoc. Off. Anal. Chem.
19. URQUIZO, S. (1969). Identification of some aromatic amines and phenolic compounds by thin-layer chromatography. Ann. Fals. Expert. Chim. **62,** 27–31.
20. ZALAZNA, K., and LEGATOWA, B. (1971). Identification of basic dyes in emulsified hair dyes by thin layer chromatography. Rocz. Panstw. Zakl. Hig. **22,** 427–30.
21. IORDANOVA, I. (1978). Quantitative determination of oxidative dyes in the hygienic evaluation of hair coloring cosmetic agents. Khig. Zdraveopaz. **21,** 83–7.
22. LEGATOWA, B. (1973). Determination of aromatic amines and aminophenols in hair dyes. Rocz. Panstw. Zakl. Hig. **24,** 393–402.
23. CORBETT, J.F., and MENKART, J. (1973). Hair coloring. Cutis **12**:190–7.
24. MARKLAND, W.R. (1966). Hair preparations. In: *Kirk-Othner Encyclopedia of Chemical Technology*, 2nd ed. New York: Interscience Publishers, Vol. 10.
25. NATIONAL CANCER INSTITUTE (NCI). (1979). Bioassay of 2-nitro-*p*-phenylenediamine for possible carcinogenicity. PB 290 304. Springfield, VA: National Technical Information Service (NTIS).
26. NCI. (1979). Bioassay of 4-nitro-*o*-phenylenediamine for possible carcinogenicity. PB 290 306. Springfield, VA: NTIS.
27. CORBETT, J.F. (1976). Hair dyes – Their chemistry and toxicology. Cosmet. Toilet. **91,** 21–8.
28. MARZULLI, F.N., GREEN, S., and MAIBACH, H.I. (1978). Hair dye toxicity – A review. J. Environ. Pathol. Toxicol. **1,** 509–30.
29. SPOOR, H.J. (1976). Semipermanent hair color. Cutis **18,** 506, 508.
30. BROWN, K. (1982). Hair colorants. J. Soc. Cosmet. Chem. **33,** 375–83.
31. RADOMSKI, J.L. (1979). The primary aromatic amines: their biological properties and structure-activity relationships. Ann. Rev. Pharmacol. Toxicol. **19,** 129–57.

*Available upon request: Administrator, Cosmetic Ingredient Review, 1110 Vermont Ave., NW, Suite 810, Washington, DC 20005.

32. MARZULLI, F.N., ANJO, D.M., and MAIBACH, H.I. (1981). In vivo skin penetration studies of 2,4-toluenediamine, 2,4-diaminoanosile, 2-nitro-*p*-phenylenediamine, *p*-dioxane and N-nitrosodiethanolamine in cosmetics. Food Cosmet. Toxicol. **19,** 743–7.
33. FOOD AND DRUG ADMINISTRATION (FDA). (December 22, 1981). Cosmetic product formulation data: (a) ingredients used in each product category, and (b) number of brand name products in each product code. Two computer printouts. Washington, DC.
34. CONSUMER REPORTS. (1979). Are hair dyes safe? pp. 456–60.
35. MENKART, J., and LANMAN, B.M. (1977). Cancer and hair dyes. NY State J. Med. **77,** 366.
36. FEDERAL REGISTER. (January 8, 1982). Phenylenediamines, response to Interagency Testing Committee **47,** 973–83.
37. THE SOCIETY OF DYERS AND COLOURISTS. (1971). *Color Index,* 3rd ed. Yorkshire, UK, Vol. 3.
38. HOCKENHULL, D.J.D., and FLOODGATE, G.D. (1952). *o*-Phenylenediamine and 1,2-diamino-4-nitrobenzene as reagents for α-keto acids. Biochem. J. **52,** 38–40.
39. TAYLOR, K.W., and SMITH, M.J.H. (1955). 1,2,-Diamino-4-nitrobenzene as a reagent for the detection and determination of α-keto acids in blood and urine. Analyst **80,** 607–13.
40. BOURGEOIS, C.F., CZORNOMAZ, A.M., GEORGE, P., BELLIOT, J.P., MAINGUY, P.R., and WATIER, B. (1975). Specific determination of vitamin C (ascorbic and dehydroascorbic acids) in foods. Analysis **3,** 540–8.
41. BOURGEOIS, C.F., and MAINGUY, P.R. (1975). Determination of vitamin C (ascorbic and dehydroascorbic acids) in foods and feeds. Int. J. Vit. Nutr. Res. **45,** 70–84.
42. LAMBERT, J.L., CHEJLAVA, M.J., PAUKSTELIS, J.V., and LIU, A.T. (1978). 4-Nitro-1,2-diaminobenzene as a new chromagen in the West-Gaeke method for sulfur dioxide. Anal. Chim. Acta **99,** 379–82.
43. CAPPON, C.J., and SMITH, J.C. (1978). Determination of selenium in biological materials by gas chromatography. J. Anal. Toxicol. **2,** 114–20.
44. SHIMOISHI, Y. (1976). The gas-chromatographic determination of selenium (VI) and total selenium in milk, milk products and albumin with 1,2-diamino-4-nitrobenzene. Analyst **101,** 298–305.
45. NAKAO, M., and TAKEDA, Y. (1983). Distribution, excretion, and metabolism of nitro-*p*-phenylenediamine in rats. J. Toxicol. Environ. Health **11,** 93–100.
46. HOWES, D., and BLACK. J.G. (1983). Percutaneous absorption of 2-nitro-*p*-phenylenediamine. Int. J. Cosmet. Sci. **5,** 215–26.
47. WERNICK, T., LANMAN, B.M., and FRAUX, J.L. (1975). Chronic toxicity, teratologic, and reproduction studies with hair dyes. Toxicol. Appl. Pharmacol. **32,** 450–60.
48. AMES, B.N., McCANN, J., and YAMASAKI, E. (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res. **31,** 347–64.
49. AMMENHEUSER, M.M., and WARREN, M.E. (1979). Detection of mutagens in the urine of rats following topical application of hair dyes. Mutat. Res. **66,** 241–5.
50. DURSTON, W.E., and AMES, B.N. (1974). A simple method for the detection of mutagens in urine: studies with the carcinogen 2-acetylaminofluorene. Proc. Natl. Acad. Sci. USA **71,** 737–41.
51. BURNETT, C., LOEHR, R., and CORBETT, J. (1977). Dominant lethal mutgenicity study on hair dyes. J. Toxicol. Environ. Health **2,** 657–62.
52. GLOXHUBER, C., POTOKAR, M., REESE, G., and FLEMMING, P. (1972). Toxikologische prufung direktziehender haarfarbstoffe. J. Soc. Cosmet. Chem. **23,** 259–69.
53. LLOYD, G.K., LIGGETT, M.P., KYNOCH, S.R., and DAVIES, R.E. (1977). Assessment of the acute toxicity and potential irritancy of hair dye constituents. Food Cosmet. Toxicol. **15,** 607–10.
54. HODGE, H.C., and STERNER, J.H. (1949). Tabulation of toxicity classes. Am. Ind. Hyg. A. Quart. **10,** 93–6.
55. REZNIK, G., and WARD, J.M. (1979). Carcinogenicity of the hair dye component 2-nitro-*p*-phenylenediamine: Induction of eosinophilic hepatocellular neoplasms in female B6C3F1 mice. Food Cosmet. Toxicol. **17,** 493–500.
56. FAIRCHILD, E.J. (ed.). (1977). *NIOSH Registry of Toxic Effects of Chemical Substances.* Rockville, MD: Tracor Jitco, Vol. II.
57. CTFA. (May 1967). Submission of data by CTFA (2-11-101). 2NPPD dermal toxicity study in rabbits.*
58. CTFA. (April 1967). Submission of data by CTFA (2-11-63). 2NPPD dermal toxicity study in rabbits.*
59. COSMETIC, TOILETRY AND FRAGRANCE ASSOCIATION (CTFA). (February 1973). Submission of data by CTFA (2-11-102). 4NOPD dermal toxicity study in rabbits.*
60. CTFA. (February 1973). Submission of data by CTFA (2-11-62). 4NOPD dermal toxicity study in rabbits.*
61. BURNETT, C., GOLDENTHAL, E.I., HARRIS, S.B., WAZETER, F.X., STRAUSBURG, J., KAPP, R., and VOELKER, R. (1976). Teratology and percutaneous toxicity studies on hair dyes. J. Toxicol. Environ. Health **1,** 1027–40.

62. SEARLE, C.E., and JONES, E.L. (1977). Effects of repeated applications of two semipermanent hair dyes to the skin of A and DBAf mice. Br. J. Cancer **36,** 467–78.
63. BURNETT, C., JACOBS, M.M., SEPPALA, A., and SHUBIK, P. (1980). Evaluation of the toxicity and carcinogenicity of hair dyes. J. Toxicol. Environ. Health **6,** 247–57.
64. INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION (IRDC). (February 6, 1979). Submission of data by CTFA (2-11-38). Lifetime toxicity/carcinogenesis study in rats.*
65. MORIKAWA, F., FUJII, S., TEJIMA, M., SUGIYAMA, H., and UZUKA, M. (1976). Safety evaluation of hair cosmetics. In: Toda, K. (ed.). *Biology and Disease of the Hair.* Baltimore, MD: University Park Press, pp. 641–57.
66. CTFA. (May 24, 1976). Submission of data by CTFA (2-11-47). Schultz, K.H. Comparable studies of sensitization of different hair dye ingredients.*
67. SHEU, C.W., and GREEN, S. (1978). Dominant lethal studies in rats of five hair dye components: 2-nitro-*p*-phenylenediamine, 4-nitro-*o*-phenylenediamine, *m*-phenylenediamine, 2,4-diaminoanisole sulfate, and 2,5-diaminoanisole sulfate. Toxicol. Appl. Pharmacol. **45,** 219.
68. SHEU, C.J., and GREEN, S. (1979). Dominant lethal assay of some hair-dye components in random-bred male rats. Mutat. Res. **68,** 85–98.
69. MARKS, T.A., GUPTA, B.N., LEDOUK, T.A., and STAPLES, R.E. (1981). Teratogenic evaluation of 2-nitro-*p*-phenylenediamine, 4-nitro-o-phenylenediamine, and 2,5-toluenediamine sulfate in the mouse. Teratology **24,** 253–65.
70. MARKS, T.A., WORTHY, W.C., and STAPLES, R.E. (1979). Teratogenicity of 4-nitro-1,2-diaminobenzene (4NDB) and 2-nitro-1,4-diaminobenzene (2NDB) in the mouse. Teratology **19,** 37A–38A.
71. IRDC. (November 2, 1977). Submission of data by CTFA. (2-11-37). Multigeneration reproduction study in rats.*
72. BIODYNAMICS. (September 7, 1977). Submission of data by CTFA (2-11-35). Project No. 76-1667. A modified segment II teratology study of hair dyes in mice.*
73. BIODYNAMICS. (1982). Submission of data by CTFA (2-11-36). Project No. 76-1666. A modified segment II teratology study of hair dyes in rabbits.*
74. AMES, B.N., KAMMEN, H.O., and YAMASAKI, E. (1975). Hair dyes are mutagenic: identification of a variety of mutagenic ingredients. Proc. Natl. Acad. Sci. USA **72,** 2423–7.
75. BRUCE, W.R., and HEDDLE, J.A. (1979). The mutagenic activity of 61 agents as determined by the micronucleus, *Salmonella,* and sperm abnormality assays. Can. J. Genet. Cytol. **21,** 319–33.
76. BYEON, W.H., PAIK, S.G., and LEE, S.Y. (1975). Mutagenicity of phenylenediamines and their derivatives. Korean J. Microbiol. **13,** 51–8.
77. CLINE, J.C., and McMAHON, R.E. (1977). Detection of chemical mutagens, use of concentration gradient plates in a high capacity screen. Res. Commun. Chem. Pathol. Pharmacol. **16,** 523–33.
78. De GIOVANNI-DONNELLY, R. (1981). The comparative response of *Salmonella typhimurium* strains TA1538, TA98, and TA100 to various hair-dye components. Mutat. Res. **91,** 21–5.
79. DUNKEL, V.C., and SIMMON, V.F. (1980). Mutagenic activity of chemicals previously tested for carcinogenicity in the National Cancer Institute bioassay program. IARC Sci. Publ. **27,** 283–302.
80. GARNER, R.C., and NUTMAN, C.A. (1977). Testing of some azo dyes and their reduction products for mutagenicity using *Salmonella typhimurium* TA 1538. Mutat. Res. **44,** 9–19.
81. INOUE, T., MORITA, K., and KADA, T. (1981). Purification and properties of a plant desmutagenic agent for the mutagenic principle of tryptophan pyrolysate. Agric. Biol. Chem. **45,** 345–53.
82. LEVIN, D.E., YAMASAKI, E., and AMES, B.N. (1982). A new *Salmonella* tester strain, TA97, for the detection of frameshift mutagens: a run of cytosines as a mutational hot-spot. Mutat. Res. **94,** 315–30.
83. MOHN, G.R., and DE SERRES, F.J. (1976). On the mutagenic activity of some hair dyes. Mutat. Res. **38,** 116–7.
84. PROBST, G.S., McMAHON, R.E., HILL, L.E., THOMPSON, C.Z., EPP, J.K., and NEAL, S.B. (1981). Chemically induced unscheduled DNA synthesis in primary rat hepatocyte cultures: a comparison with bacterial mutagenicity using 218 compounds. Environ. Mutagen. **3,** 11–32.
85. SEARLE, C.E., and HARNDEN, D.G. (1975). Tests of two hair colourants for carcinogenicity by repeated application to mouse skin. Br. J. Cancer **32,** 251.
86. SHAHIN, M.M., and VON BORSTEL, R.C. (1976). Comparative studies of six compounds in *Saccharomyces cerevisiae* and *Salmonella typhimurium* reversion studies. Mutat. Res. **38,** 379–80.
87. SHAHIN, M.M., and VON BORSTEL, R.C. (1978). Comparison of mutation induction in reversion systems of *Saccharomyces cerevisiae* and *Salmonella typhimurium.* Mutat. Res. **53,** 1–10.
88. YOSHIKAWA, K., UCHINO, H., and KURATA, H. (1976). Studies on the mutagenicity of hair dye. Eisei Shikensho Hokoku **94,** 28–32.

89. YOSHIKAWA, K., UCHINO, H., TATENO, N., and KURATA, H. (1977). Mutagenic activities of the samples prepared with raw material of hair dye. Eisei Shikensho Hokoku **95,** 15–24.
90. DE SERRES, F.J., and SHEBLY, M.D. (1979). Recommendations on data production and analysis using the *Salmonella*/microsome-mutagenicity assay. Mutat. Res. **64,** 159–65.
91. AESCHBACHER, H.U., and RUCH, E. (1982). Urine-mediated Ames test: interactions. Mutat. Res. **103,** 127–31.
92. BENIGNI, R., BIGNAMI, M., CARERE, A., CONTI, G., CONTI, L., CREBELLI, R., DOGLIOTTI, E., GUALANDI, G., NOVELLETTO, A., and ORTALI, V.A. (1979). Mutational studies with diquat and paraquat in vitro. Mutat. Res. **68,** 183–93.
93. COLEMAN, N., GARDNER, A., and HERBERT, V. (1979). Nonmutagenicity of gossypol in the *Salmonella*/mammalian-microsome plate test. Environ. Mutagen. **1,** 315–20.
94. SUMMER, K., and GOEGGELMANN, W. (1980). 1-Chloro-2,4-dinitrobenzene depletes glutathione in rat skin and is mutagenic in *Salmonella typhimurium.* Mutat. Res. **77,** 91–3.
95. SUMMER, K., and GOEGGELMANN, W. (1980). Mutagenicity of 1-fluoro-2,4-dinitrobenzene is affected by bacterial glutathione. Mutat. Res. **70,** 173–8.
96. ZEIGER, E., PAGANO, D.A., and ROBERTSON, I.G. (1981). A rapid and simple scheme for confirmation of *Salmonella* tester strain phenotype. Environ. Mutagen. **3,** 205–9.
97. SEARLE, C.E., HARNDEN, D.G., VENITT, S., and GYDE, O.H.B. (1975). Carcinogenicity and mutagenicity tests of some hair colorants and constituents. Nature **255,** 506–7.
98. VENITT, S., and SEARLE, C.E. (1976). Mutagenicity and possible carcinogenicity of hair colorants and constituents. In: *International Agency for Research on Cancer, IARC Scientific Publications No. 13.* Lyon, France: INSERM Symposia Series, Vol. 52, pp. 263–72.
99. BURNETT, C.M., FUCHS, C.M., and CORBETT, J.F. (1979). Mutagenicity studies on urine concentrates from female users of dark hair color products. Drug Chem. Toxicol. **2,** 283–93.
100. CTFA. (January 31, 1983). Summary of cosmetic ingredient safety analysis on 2-nitro-*p*-phenylenediamine and 4-nitro-*o*-phenylenediamine.*
101. McMAHON, R.E., CLINE, J.C., and THOMPSON, C.Z. (1979). Assay of 855 test chemicals in ten tester strains using a new modification of the Ames test for bacterial mutagens. Cancer Res. **39,** 682–92.
102. VENITT, S. (1978). Mutagenicity of hair dyes: some more evidence and the problems of its interpretation. Mutat. Res. **53,** 278–9.
103. TWEATS, D.J., GREEN, M.H.L., and MURIEL, W.J. (1981). A differential killing assay for mutagens and carcinogens based on an improved repair-deficient strain of *Escherichia coli.* Carcinogenesis **2,** 189–94.
104. VENITT, S., and BUSHELL, C.T. (1975). Mutagenicity of hair colorants in bacteria: possible link with carcinogenicity. Br. J. Cancer **32,** 251.
105. McCARROLL, N.E., KEECH, B.H., and PIPER, C.E. (1981). A microsuspension adaptation of the *Bacillus subtilis rec* assay. Environ. Mutagen. **3,** 607–16.
106. MacGREGOR, J.T., and SACKS, L.E. (1979). The *Bacillus subtilis* multigene sporulation test: Sensitivity to known mutagens and carcinogens. Environ. Mutagen. **1,** 121.
107. SACKS, L.E., and MacGREGOR, J.T. (1982). The *B. subtilis* multigene sporulation test for mutagens: detection of mutagens inactive in the *Salmonella* his reversion test. Mutat. Res. **95,** 191–202.
108. MAYER, V.W., and GOIN, C.J. (1980). Induction of mitotic recombination by certain hair-dye chemicals in *Saccharomyces cerevisiae.* Mutat. Res. **78,** 243–52.
109. ONG, T. (1978). Use of the spot, plate, and suspension test systems for the detection of the mutagenicity of environmental agents and chemical carcinogens in *Neurospora crassa.* Mutat. Res. **53,** 297–308.
110. BIGNAMI, M., CARERE, A., CONTI, G., CONTI, L., CREBELLI, R., and FABRIZI, M. (1982). Evaluation of 2 different genetic markers for the detection of frameshift and missense mutagen in *A. nidulans.* Mutat. Res. **97,** 293–302.
111. PALMER, K.A., DENUNZIO, A., and GREEN, S. (1976). The mutagenic assay of some hair dye components using the thymidine kinase locus of L51178Y mouse lymphoma cells. Toxicol. Appl. Pharmacol. **37,** 108.
112. PALMER, K.A., DENUNZIO, A., and GREEN, S. (1978). The mutagenic assay of some hair dye components, using the thymidine kinase locus of L5178Y mouse lymphoma cells. J. Environ. Pathol. Toxicol. **1,** 87–91.
113. NATIONAL TOXICOLOGY PROGRAM. (July 1982). NTP Technical Bulletin No. 8.
114. BLIJLEVEN, W.G.H. (1977). Mutagenicity of four hair dyes in *Drosophila melanogaster.* Mutat. Res. **48,** 181–5.
115. HUANG, S.L. (1977). The mutability of the *Minute* loci and sex-linked recessive lethals in *Drosophila melanogaster.* Mutat. Res. **44,** 145–8.

116. FAHMY, M.J., and FAHMY, O.G. (1977). Mutagenicity of hair dye components relative to the carcinogen benzidine in *Drosophila melanogaster*. Mutat. Res. **56,** 31–7.
117. HEDDLE, J.A., and BRUCE, W.R. (1977). Comparison of tests for mutagenicity or carcinogenicity using assays for sperm abnormalities, formation of micronuclei, and mutations in *Salmonella*. Cold Spring Harbor Conf. Cell Prolif. **4C,** 1549–57.
118. BENEDICT, W.F. (1976). Morphological transformation and chromosome aberrations produced by two hair dye components. Nature **260,** 368–9.
119. ISHIDATE, M., and ODASHIMA, S. (1977). Chromosome tests with 134 compounds on Chinese hamster cells in vitro—A screening for chemical carcinogens. Mutat. Res. **48,** 337–53.
120. ISHIDATE, M., and YOSHIKAWA, K. (1980). Chromosome aberration tests with Chinese hamster cells in vitro with and without metabolic activation: a comparative study on mutagens and carcinogens. In: Further studies in the assessment of toxic actions. Arch. Toxicol. [Suppl.] **4,** 41–4.
121. KIRKLAND, D.J., and VENITT, S. (1976). Cytotoxicity of hair colorant constituents. Chromosome damage induced by two nitrophenylenediamines in cultured Chinese hamster cells. Mutat. Res. **40,** 47–55.
122. PERRY, P.E., and SEARLE, C.E. (1977). Induction of sister chromatid exchanges in Chinese hamster cells by the hair dye constituents 2-nitro-*p*-phenylenediamine and 4-nitro-*o*-phenylenediamine. Muat. Res. **56,** 207–10.
123. HOSSACK, D.J.N., and RICHARDSON, J.C. (1977). Examination of the potential mutagenicity of hair dye constituents using the micronucleus test. Experientia **33,** 377–8.
124. WILD, D., KING, M.T., and ECKHARDT, K. (1980). Cytogenetic effect of ortho-phenylenediamine in the mouse, Chinese hamster, and guinea pig and of derivatives, evaluated by the micronucleus test. Arch. Toxicol. **43,** 249–55.
125. GRIFFITHS, A.J.F. (1979). *Neruospora* prototroph selection system for studying aneuploid production. Environ. Health Perspec. **31,** 75–80.
126. PAINTER, R.B., and HOWARD, R. (1982). The HeLa DNA synthesis inhibition test as a rapid screen for mutaenic carcinogens. Mutat. Res. **92,** 427–38.
127. MARTIN, C.N., McDERMID, A.C., and GARNER, R.C. (1978). Testing of known carcinogens and noncarcinogens for their ability to induce unscheduled DNA synthesis in HeLa cells. Cancer Res. **38,** 2621–7.
128. PROBST, G.S., and HILL, L.E. (1980). Chemically induced DNA repair synthesis in primary rat hepatocytes: A correlation with bacterial mutagenicity. Ann. NY Acad. Sci. **349,** 405–6.
129. WILLIAMS, G.M., LASPIA, M.F., and DUNKEL, V.C. (1982). Reliability of the hepatocyte primary culture/DNA repair test in testing of coded carcinogens and noncarcinogens. Mutat. Res. **97,** 359–70.
130. SMITH, N.S., BISHUN, N.P., and WILLIAMS, D. (1976). Depression of lymphocyte transformation by two hair dye constituents. Microbios Lett. **1,** 205–8.
131. PIENTA, R.J. (1980). Transformation of Syrian hamster embryo cells by diverse chemicals and correlation with their reported carcinogenic and mutagenic activities. *Chemical Mutagens: Principal Methods for Their Detection* **6,** 175–202.
132. PIENTA, R.J. (1980). Evaluation and relevance of the Syrian hamster embryo cell system. Appl. Methods Oncol. **3,** 149–69.
133. PIENTA, R.J., and KAWALEK, J.C. (1981). Transformation of hamster embryo cells by aromatic amines. Natl. Cancer Inst. Monogr. **58,** 243–51.
134. TRAUL, K.A., TAKAYAMA, K., KACHEVSKY, V., HINK, R.J., and WOLFF, J.S. (1981). Rapid in vitro assay for carcinogenicity of chemical substances in mammalian cells utilizing an attachment-independence endpoint. 2. Assay validation. J. Appl. Toxicol. **1,** 190–5.
135. YOSHIKURA, H., KUCHINO, T., and MATSUSHIMA, T. (1979). Carcinogenic chemicals enhance mouse leukemia virus infection in contact-inhibited culture: a new simple method of screening carcinogens. Cancer Lett. **7,** 203–8.
136. CTFA. (December 17, 1981). Submission of data by CTFA (2-11-4). CTFA interoffice correspondence and letters on NCI study by two pathologists.*
137. NORTH AMERICAN CONTACT DERMATITIS GROUP. (December 4, 1980). Standard screening tray, 1979 vs. 1980 summary.
138. DERMA-TEST LABORATORIES (DTL). (September 22, 1982). Submission of data by CTFA. Repeated insult patch test, product: 1101-49 and 1101-52 mixed equal parts.*
139. DTL. (September 22, 1982). Submission of data by CTFA. Repeated insult patch test, product: 1101-48 and 1101-52 mixed equal parts.*
140. DTL. (September 22, 1982). Submission of data by CTFA. Repeated insult patch test, product: 1101-50 and 1101-52 mixed equal parts.*
141. HINDSON, C. (1975). *o*-Nitro-paraphenylenediamine in hair dye—An unusual dental hazard. Contact Dermatitis **1,** 333.

142. ANTHONY, H.M., and THOMAS, G.M. (1970). Tumors of the urinary bladder: An analysis of the occupations of 1030 patients in Leeds, England. J. Natl. Cancer Inst. **45,** 879–98.
143. COLE, P., HOOVER, R., and FRIEDALL, C.H. (1972). Occupation and cancer of the lower urinary tract. Cancer **29,** 1250–60.
144. DUNHAM, L.J., RABSON, A.S., STEWART, H.L., FRANK, A.S., and YOUNG, J.L. (1968). Rate, interview, and pathology study of cancer of the urinary bladder in New Orleans, LA. J. Natl. Cancer Inst. **41,** 683–709.
145. WYNDER, E.L., ONDERDONK, J., and MANTEL, N. (1963). An epidemiological investigation of cancer of the bladder. Cancer **16,** 1388–407.
146. GARFINKEL, J., SELVIN, S., and BROWN, S.M. (1977). Possible increased risk of lung cancer among beauticians. J. Natl. Cancer Inst. **58,** 141–3.
147. MENCK, H.R., PIKE, M.C., HENDERSON, B.E., and JING, J.S. (1977). Lung cancer risk among beauticians and other female workers. J. Natl. Cancer Inst. **59,** 1423–5.
148. JAIN, M., MORGAN, R.W., and ELINSON, L. (1977). Hair dyes and bladder cancer. Can. Med. Assoc. J. **117,** 1131–3.
149. HENNEKENS, C.H., SPEIZER, F.E., ROSNER, B., BAIN, C.J., BELANGER, C., and PETO, R. (1979). Use of permanent hair dyes and cancer among registered nurses. Lancet **1**:1390–3.
150. KINLEN, L.J., HARRIS, R., GARROD, A., and RODRIGUEZ, K. (1977). Use of hair dyes by patients with breast cancer: A case-control study. Br. Med. J. **2,** 366–8.
151. NASCA, P.C., LAWRENCE, C.E., GREENWALD, P., CHOROST, S., ARBUCKLE, J.T., and PAULSON, A. (1979). Relationship of hair dye use, benign breast disease, and breast cancer. J. Natl. Cancer Inst. **64,** 23–8.
152. SHAFER, N., and SHAFER, R.W. (1976). Potential of carcinogenic effects of hair dyes. NY State J. Med. **76,** 394–6.
153. SHORE, R.E., PASTERNACK, B.S., THIESSEN, E.U., SADOW, M., FORBES, R., and ALBERT, R.E. (1979). A case-control study of hair dye use and breast cancer. J. Natl. Cancer Inst. **62,** 277–83.
154. WYNDER, E.L., and GOODMAN, M. (1983). Epidemiology of breast cancer and hair dyes. J. Natl. Cancer Inst. **71,** ???.
155. FEDERAL REGISTER. (October 16, 1979). Cosmetic product warning statements coal tar hair dyes containing 4-methoxy-*m*-phenylenediamine (2,4-diaminoanisole) or 4-methoxy-*m*-phenylenediamine (2,4,-diaminoanisole sulfate). **44,** 59509–22.
156. CLEMMESEN, J. (1981). Epidemiological studies into the possible carcinogenicity of hair dyes. Mutat. Res. **87,** 65–79.