Safety Assessment of *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate as Used in Cosmetics

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*All interested persons are provided 60 days from the above release date (i.e., **March 8, 2024**) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party, and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.*

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ABBREVIATIONS

ADME	absorption, distribution, metabolism, and excretion
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the curve
C_{max}	peak concentration
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
CI	confidence interval
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPK	creatine phosphokinase
CPPD	*N*-phenyl-*N*'-cyclohexyl-*p*-phenylenediamine
CPSC	Consumer Product Safety Commission
CYP	cytochrome P450
DAPPD	*N*,*N*-diacetyl-*p*-phenylenediamine
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
DMSO	dimethyl sulfoxide
dpm	disintegration per min
EC_3	estimated concentrations of an SI of 3
ECHA	European Chemicals Agency
ED_{10}	threshold value for 10%
FDA	Food and Drug Administration
FD&C	Food, Drug and Cosmetic
GIRDCA	Gruppo Italiano Ricerca Dermatiti da Contatto e Ambientali
HPLC	high-performance liquid chromatography
HRIPT	human repeated insult patch test
IARC	International Agency for Research on Cancer
IC_{50}	inhibitory concentration of 50%
ICDRG	International Contact Dermatitis Research Group
IPPD	*N*-isopropyl-*N*'-phenyl-*p*-phenylenediamine
IVDK	Information Network of Departments of Dermatology
LDH	lactate dehydrogenase
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect level
MAPPD	monoacetyl-*p*-phenylenediamine
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
NACDG	North American Contact Dermatitis Group
NAT	*N*-acetyltransferase
NCI	National Cancer Institute
NIOSH	National Institute for Occupational Safety and Health
NOEL	no-observed-effect level
NOAEL	no-observed-adverse-effect level
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio
OSHA	Occupational Safety and Health Administration
Panel	Expert Panel for Cosmetic Ingredient Safety
PEL	permissible exposure limit
pet.	petrolatum
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
REL	recommended exposure limit
ROS	reactive oxygen species
SCCNFP	Scientific Committee on Cosmetic and Non-Food Products
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dose
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SI	stimulation index
T_{max}	time-to-peak concentration
TC_{50}	50% toxic concentration

TG	test guideline
TRUE	thin-layer rapid-use epicutaneous
TWA	time-weighted average
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate, which are reported to function as hair dyes in cosmetic products. The Panel reviewed the available data to determine the safety of these ingredients. The Panel concluded that *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate are safe for use as hair dye ingredients in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This assessment reviews the safety of *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook (*wINCI; *Dictionary*; see Table 1*)*, these ingredients function as hair colorants in cosmetic products.[1]

The Expert Panel for Cosmetic Safety (Panel) first reviewed the safety of *p*-Phenylenediamine individually in a report published in 1985, with the conclusion "*p*-Phenylenediamine is a known sensitizer, and some persons may be sensitized under intended conditions of use. For those persons not sensitized, the Expert Panel concludes that *p*-Phenylenediamine is safe as a hair dye ingredient at the current concentrations of use."[2] This conclusion was reaffirmed in a re-review that was published in 2006.[3]

Subsequently, the *p*-Phenylenediamine report was reopened to add *p*-Phenylenediamine HCl and *p*-Phenylenediamine Sulfate. That amended report was finalized in 2007 with the conclusion that these 3 ingredients are safe as hair dyes in the practices of use and concentration as described in the safety assessment, but it was never published.[4] Accordingly, this current amended report is an updated version of the 2007 assessment, and includes all studies considered in the 2007 amended report as well as studies published since then. Additionally, excerpts from the summaries of the 1985 report are disseminated throughout the text of this re-review document, as appropriate, and are identified by *italicized text*.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; this search was last performed October 2023. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Some chemical and toxicological data on *p*-Phenylenediamine and the hydrochloride and sulfate salts included in this safety assessment were obtained from robust summaries of data submitted to the European Chemicals Agency (ECHA) by companies as part of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) chemical registration process.[5,6] Additionally, data were obtained from opinions produced by the Scientific Committee on Cosmetic and Non-Food Products (SCCNFP), Scientific Committee on Consumer Products (SCCP), and Scientific Committee on Consumer Safety (SCCS) of the European Commission.[7-9] These data summaries are available on the databases for ECHA and the European Commission, respectively, and when deemed appropriate, information from the summaries has been included in this report.

CHEMISTRY

Definition and Structure

The definitions and structures of *p*-Phenylenediamine (CAS No.106-50-3), *p*-Phenylenediamine HCl (CAS No. 624-18-0) and *p*-Phenylenediamine Sulfate (CAS No. 16245-77-5; 50994-40-6) are provided in Table 1.[1]

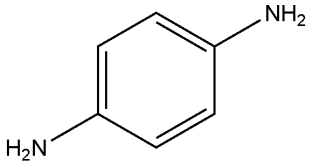


Figure 1. *p*-Phenylenediamine

In the permanent hair dyeing process, *p*-Phenylenediamine acts as the primary intermediate or precursor in formulation with a coupling agent (e.g., resorcinol) in an alkaline medium (ammonia) prior to mixing with a hydrogen peroxide solution.[10,11] Under the alkaline conditions in the presence of hydrogen peroxide, *p*-Phenylenediamine is oxidized to produce quinone diimine, which reacts with the coupling agent to form the leuco dye. This is then converted into the indoaniline dye within the hair strand.

Chemical Properties

Aromatic amines, such as p-Phenylenediamine, are nonpolar bases that are readily converted to highly water-soluble hydrochloride salts.[2] Whereas the salts of aromatic amines are relatively stable, free aromatic amines are usually quite unstable to light, heat, and oxygen and oxidize to colored quinoneimines, quinones, and various polymerized products. When used in hair dyes, the amines are usually mixed with hydrogen peroxide immediately before use, producing the oxidation products. The oxidation products then react with sulfhydryl groups present in the hair to form permanent bonds.

p-Phenylenediamines and their oxidation products are highly reactive substances that would be expected to react with tissue nucleophiles, causing various biological effects. Aromatic amines can undergo both N-hydroxylation and ring epoxidation. N-Hydroxylation and epoxidation are steps in the metabolic activation of aromatic hydrocarbons to mutagens and carcinogens. Phenylenediamine compounds are also potent antioxidants.

p-Phenylenediamine occurs in the form of white to light purple monoclinic crystals. It is soluble in water, alcohol, ether, benzene, chloroform, and acetone and is insoluble in caustic soda. The compound reacts with oxidizing materials. On exposure to air, p-Phenylenediamine oxidizes to form a purple or black color. Brown and black colors can also develop when the compound is exposed to 5% iron (III) chloride and 3% hydrogen peroxide solutions, respectively. Quinoneimine compounds resulting from the oxidation of p-Phenylenediamine may hydrolyze in aqueous media to yield p-benzoquinone and ammonia. P-Phenylenediamine is combustible and, when heated, emits highly toxic fumes of nitrogen compounds. Degradation following exposure to activated sludge microorganisms has also been reported.

Chemical properties for *p*-Phenylenediamine and the related hydrochloride and sulfate salts are summarized in Table 2. The estimated and experimental log P_{ow} values for *p*-Phenylenediamine are -0.31 and -0.86, respectively.

Method of Manufacture

p-Phenylenediamine is prepared by reducing p-dinitrobenzene with iron and hydrochloric acid, or by reducing p-nitroaniline with the one of the following: iron and hydrochloric acid; iron, ammonium polysulfide and hydrogen; or iron and ferrous chloride.[2] The resulting material can be purified by crystallization.

Additional data indicate that p-Phenylenediamine is manufactured using the following methods: reduction of p-nitroaniline, aniline diazotization, and direct nitration of benzene without chlorinating.[4] The third method does not lend itself to and has not been shown to contain chlorinated compounds such as chloro- and dichloroanilines or aminobiphenyls.

Impurities

p-Phenylenediamine produced in the US has a purity of > 99% for use in hair dyes via the process of direct nitration of benzene without chlorinating.[4]

p-Phenylenediamine

According to the SCCP, the purity of *p*-Phenylenediamine (determined by high-performance liquid chromatography (HPLC)) is > 99%.[7] Impurities of *p*-Phenylenediamine (reported as specification limits) include *o*-aminophenol (< 500 ppm), *o*-phenylenediamine (< 200 ppm), *m*-phenylenediamine (< 200 ppm), and aniline (< 50 ppm). Content analysis of 4 batches of *p*-Phenylenediamine reported the impurity content as 190 – 400 µg/g *o*-aminophenol, < 10 – 120 µg/g *o*-phenylenediamine, 65 – 140 µg/g *m*-phenylenediamine, and 50 – 100 µg/g aniline. Solvent content was reported to be < 100 ppm. Heavy metal content was < 5 ppm each for mercury, arsenic, and antimony, < 10 ppm cadmium, and < 20 ppm lead.

p-Phenylenediamine HCl

According to the SCCP, the relative purity of *p*-Phenylenediamine HCl (determined by HPLC) is > 99%.[7]

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics, and does not cover their use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to the 2023 VCRP survey data, *p*-Phenylenediamine is reported to be used in 200 formulations (Table 3).[12] The majority of these uses are in hair coloring preparations; however, 7 uses have been reported for eye makeup preparations.

With regard to the reported use in eye makeup preparations, the US Federal Food, Drug and Cosmetic Act (FD&C Act) mandates that color additives must be approved by FDA for their intended use before they are used. Additionally, the use of *p*-Phenylenediamine in dark (black) henna tattoos/temporary tattoos has been reported through multiple case studies of adverse reactions (see Case Reports Related to Temporary Tattooing further on in this report). *p*-Phenylenediamine is an unapproved color additive in cosmetics products, and thereby, such use is not permitted. These uses are not within the purview of this Panel.

Only 1 use was reported in a hair coloring shampoo for *p*-Phenylenediamine HCl and no uses were reported for the sulfate salt. The frequencies of use for *p*-Phenylenediamine have greatly decreased since the initial amended report was finalized; in 2007, *p*-Phenylenediamine was reported to have 1497 uses, all in hair coloring formulations.[4] No uses were reported at that time for the related salts.

The results of the concentration of use survey conducted by the Council in 2022 indicate *p*-Phenylenediamine has a maximum concentration of use range of 0.98 - 3% in hair dyes, with a maximum on-head concentration after dilution of 1%.[13] No concentrations of use were reported for related salts. In the 2007 amended report, the maximum concentration of uses range for *p*-Phenylenediamine was 2 - 4% in hair dyes; the hydrochloride salt and the sulfate salt were each reported to be used at a maximum concentration of 6% in hair dyes.[4]

Although products containing this ingredient may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of this ingredient (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

This ingredient is considered a coal tar hair dye for which regulations require caution statements and instructions regarding patch tests in order to be exempt from certain adulteration and color additive provisions of the FD&C Act. In order to be exempt, the following caution statement must be displayed on all coal tar hair dye products:

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

Product labels shall also bear patch test instructions for determining whether the product causes skin irritation. However, whether or not patch testing prior to use is appropriate is not universally agreed upon. The Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 h after application of the test material and prior to the use of a hair dye formulation. Conversely, a report in Europe suggests that self-testing has severe limitations, and may even cause morbidity in consumers.[14,15] Hair dye products marketed and sold in the US, though, must follow the labeling requirements established by the FD&C Act.

Under European regulations for cosmetic ingredients, *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate are listed in Annex III with the restrictions that these ingredients may be used at only up to 2% (free base) in oxidizing hair dyes.[16] Additionally, *p*-Phenylenediamine and its hydrochloride and sulfate salts may be used in products intended for coloring eyelashes after mixing under oxidative conditions and the maximum concentration applied to eyelashes must not exceed 2% (free base); application is for professional use only.

Based on a toxicokinetic-based approach margin of safety calculation, exposure time, and exposure type (mainly to a non-mutagenic, detoxified metabolite), the SCCS expressed no concern regarding systemic toxicity from use of *p*-Phenylenediamine in oxidative hair dyes at on-head concentrations of up to 2.0%.[8] Further, the SCCS has determined that no conclusion with regard to carcinogenicity of *p*-Phenylenediamine as used in oxidative hair dye formulations can be drawn. However, based on toxicokinetic and genotoxicity data, the SCCS decided it was unlikely that *p*-Phenylenediamine as used in hair dye formulations would pose a carcinogenic risk for consumers. Additionally, the SCCS found that *p*-Phenylenediamine in hair dyes remains a considerable concern for consumer safety.

Non-Cosmetic

In addition to its cosmetic use in permanent hair coloring formulations, p-Phenylenediamine is used as a photographic developing agent, a dye developer for furs, an industrial chemical intermediate, an intermediate in the preparation of antioxidants and rubber accelerators, and as an antioxidant for rubber in sewer pipe joints.[2] p-Phenylenediamine is also used in x-ray film fluids, printer's ink, clothing, shoes, leather processing, lithographic processing, photochemical measurements, rubber vulcanization, printing of cellulosic textile materials, dye stuff manufacture, and production of poly-paraphenylene terephthalamide.

Chemical and biochemical applications of p-Phenylenediamine include use as an indicator and reagent for nitrogen, as a chromogenic spray reagent for thin-layer chromatography, and as a hydrogen donor for peroxidase assay systems.[2] p-Phenylenediamine is also used for removing nitrogen and sulfur oxides from waste gases and for the calorimetric determination of hydrogen sulfide in air, thiocyanate in biological fluids, and inorganic phosphorus in serum. Other applications include use as a substrate to measure the activity of oxidative enzymes, and as a staining agent for biological materials.

The hydrochloride salt of p-Phenylenediamine is used as an analytical reagent in the testing of blood, hydrogen sulfide, amyl alcohol, and milk and as a color and pigment intermediate in fur and textile dyeing.[2] It is also used in the manufacture of rubber and plastics. Derivatives of p-Phenylenediamine are important antioxidants in synthetic and natural rubbers, petroleum products, cellulose ethers, and alfalfa meal.

TOXICOKINETIC STUDIES

p-Phenylenediamine is absorbed and excreted by both animals and humans.[2] Radioactivity was found in the blood of rabbits after the intravenous and dermal administration of [^{14}C]p-Phenylenediamine. Radioactivity was distributed throughout the body and in the blood after the intravenous and topical administration of p-Phenylenediamine to mice. In dogs, p-Phenylenediamine was found in the blood after topical and intravenous administration and was excreted in the urine after topical and subcutaneous administration. Radiolabeled [^{14}C]p-Phenylenediamine was applied topically to humans and radioactivity was found in the urine. When a hair dye containing [^{14}C]p-Phenylenediamine was used on monkeys and by humans, radioactivity was detected in the hair and in the urine. Radiolabeled [^{14}C]p-Phenylenediamine was administered to rabbits by subconjunctival injection, intravitreal injection, eyedrops, and subcutaneous injection into the head. Rapid clearance of radioactivity from the site of administration was observed. Radiolabeled p-Phenylenediamine was administered intraperitoneally to rats, and radioactivity was distributed throughout the body and excreted in the urine, feces, and bile. N,N'-diacetyl-p-phenylenediamine (DAPPD), p-aminoacetanilide, and unchanged p-Phenylenediamine were identified as urinary metabolites.

Dermal Penetration

In Vitro

p-Phenylenediamine

The percutaneous absorption of a homologous series of hair dyes (*p*-Phenylenediamine included) was studied using human epidermis from abdominal skin.[17] Circular pieces of skin were clamped between two halves of a diffusion cell and aqueous solutions of the hair dyes were applied. The volume applied (0.5 ml) completely covered the 1.13 cm^2 of exposed skin in each cell. Permeability constants were determined by dividing the steady-state absorption rate by the initial vehicle concentration of the applied compound. Octanol/water partition coefficients were determined by shaking the test compound in a mixture containing 5 ml of water and 5 ml of octanol. At the end of 24 h, the ratio of the amount of dye in each solvent was determined. An octanol/water partition coefficient of 0.5 (log P_{ow} = -0.31) and a permeability constant of 2.4 x 10^{-4} cm/h were reported for *p*-Phenylenediamine.

The percutaneous absorption potential over 48 h of [^{14}C]*p*-Phenylenediamine was evaluated under 5 different dosing conditions.[9] Franz static cells with human skin were utilized. Receptor fluid was Dulbecco's phosphate buffered saline containing antioxidant. The 5 dosing conditions were as follows:

- 100 mg/cm^2 of 1.3% *p*-Phenylenediamine and other dyes in the presence of developer, but absence of hair
- 100 mg/cm^2 of 1.3% *p*-Phenylenediamine and other dyes in the presence of developer and hair
- 100 mg/cm^2 of 2.7% *p*-Phenylenediamine, but no other dyes, developer, or hair
- 20 mg/cm^2 of 2.7% *p*-Phenylenediamine, but no other dyes, developer, or hair
- 100 mg/cm^2 of 1.3% *p*-Phenylenediamine, but no other dyes, developer, or hair

The first dosing condition included 30 cells, the remaining 4 had 15 cells each. The human hair (5 mg/cm^2) in the second dosing condition was placed on the skin surface before addition of the formulation.

The skin penetration was between 0.1 and 0.2% of the applied dose. This corresponded to a cumulative mass absorbed of about 1.9 – 2.4 µg/cm^2 for the complete dye formulations. The amount of radioactive material found in the skin itself ranged from 0.65 to 6.72 µg/cm^2 (0.04-0.5%). The maximum cumulative absorption of *p*-Phenylenediamine for all formulations occurred 4 h post-application. Permeation was concentration- and dose-related. The presence of hair on the surface did not significantly affect the permeation process. A greater amount of *p*-Phenylenediamine was found on or in the skin (but not in the receptor fluid) when it was applied in the presence of developer and other dyes and in the presence of hair. The recovery rate was determined to be between 83.6 and 104%.[9]

p-Phenylenediamine HCl

The percutaneous absorption of a commercial [^{14}C]*p*-Phenylenediamine HCl-containing oxidative hair dye was investigated using human and pig ear skin.[18] To a hair dye formulation containing 3.68% cold *p*-Phenylenediamine HCl, 0.3% of [^{14}C]*p*-Phenylenediamine HCl was directly added resulting in an isotopic formulation containing 3.98% *p*-Phenylenediamine HCl with a specific activity of 1 mCi/g. The developer was 6% hydrogen peroxide. After mixing an equal part of formulation and developer, the specific activity of the formulation applied was 999 disintegration per min (dpm)/µg. The test material (20 mg/cm^2) was applied to the human and pig ear skin samples in static diffusion cells (2 cm^2) for 0.5 h. The receptor fluid was Dulbecco modified phosphate buffered saline solution. At the end of the exposure time, the skin was washed, and the diffusion was allowed to continue for 24 h prior to radioactivity analysis.

For the human skin, the total absorbed amount was 2.4 ± 1.6% (10.6 ± 6.7 $\mu g_{eq}/cm^2$) of the applied dose. The majority of the radioactivity was recovered in the surface excess (98.8 ± 5.9%), with 1.28 ± 0.59%, 1.29 ± 0.54%, and 1.14 ± 1.15% recovered in the stratum corneum, epidermis/dermis, and receptor fluid, respectively. For the pig ear skin, the total absorbed amount was 3.4 ± 1.7% (14.6 ± 6.9 $\mu g_{eq}/cm^2$). Again, the majority of the radioactivity was recovered in the surface excess (92.4 ± 3.0%), with 1.69 ± 0.72%, 3.05 ± 1.49%, and 0.33 ± 0.19% recovered in the stratum corneum, epidermis/dermis, and receptor fluid, respectively.[18]

Human

p-Phenylenediamine HCl

The percutaneous absorption of a commercial [^{14}C]*p*-Phenylenediamine HCl-containing oxidative hair dye was investigated in 8 male volunteers.[18] The tested material was prepared as an isotopic dilution of 1.6 MBq [^{14}C]*p*-Phenylenediamine HCl with 40 ml of a commercial dark shade oxidative hair dye formulation containing 3.98% cold *p*-Phenylenediamine HCl and 2.0% *m*-aminophenol. The actual content of *p*-Phenylenediamine HCl in the hair dye formulation after isotopic dilution and prior to mixing with the developer (6% hydrogen peroxide) was about 4%. The specific activity of the formulation applied was a mean total of 7.14 ± 0.26 x 10^7 dpm, corresponding to a mean value of 1.31 ± 0.05 g_{eq} [^{14}C]*p*-Phenylenediamine HCl per volunteer, ranging from 1.22 to 1.36 g_{eq} [^{14}C]*p*-Phenylenediamine HCl.

The hair of the volunteers was cut to a standard length, dyed (30 min development), washed, dried, clipped, and collected. The hair, washing water, materials used in the study (gloves, paper towels, caps, etc.), and a 24-h scalp wash were collected for determination of radioactivity. Blood, urine, and feces were analyzed up to 120 h after hair dyeing. The recovery rate was 95.7 ± 1.5% of the applied radioactivity. The washing water, cut hair, materials used in the study, and the scalp wash contained a total of 95.16 ± 1.46% of the applied radioactivity. Absorbed radioactivity was determined to be 0.50 ± 0.24% in the urine and 0.04 ± 0.04% in the feces, which corresponds to a mean of 7.0 ± 3.4 mg_{eq} of [^{14}C]*p*-Phenylenediamine absorbed. Most of the radioactivity was eliminated within 24 h of application. The peak concentration (C_{max}) of [^{14}C]*p*-Phenylenediamine HCl in the plasma was 0.087 μg_{eq}/ml, the time-to-peak concentration T_{max} was approximately 2 h, and the mean the area under the curve $(AUC)_{0-12\ h}$ was 0.67 μg_{eq} h/ml.[18]

Absorption, Distribution, Metabolism, and Excretion (ADME)

In Vitro

p-Phenylenediamine

The capacity for *N*-acetylation of *p*-Phenylenediamine in human skin samples was investigated.[19] *p*-Phenylenediamine was acetylated to monoacetyl-*p*-phenylenediamine (MAPPD), which in turn was acetylated to DAPPD. This was determined using cytosolic fractions from human skin (n = 9) and cultured normal human epidermal keratinocytes (n = 7).

In a biotransformation study using reconstructed human epidermis and human hepatocytes, [^{14}C]*p*-Phenylenediamine was converted to MAPPD and DAPPD derivatives.[20] At higher concentrations of [^{14}C]*p*-Phenylenediamine (250 to 1000 µM), the epidermis and the hepatocytes produced more of the MAPPD. However, concentrations below 250 µM favored the formation of the DAPPD metabolite. When compared to the epidermis, the capacity of human hepatocytes for generation of MAPPD and DAPPD was 3-fold and 8-fold greater, respectively. There was no evidence that [^{14}C]*p*-Phenylenediamine was transformed to *N*-hydroxylated derivatives in the epidermis or hepatocytes.

Intact human hepatocytes, human liver microsomes, and heterologously expressed human cytochrome P450s (CYPs) were utilized to determine whether [^{14}C]*p*-Phenylenediamine is metabolized by hepatic CYPs to form an *N*-hydroxylamine.[21] Cryopreserved human hepatocytes were obtained from 4 male donors. [^{14}C]*p*-Phenylenediamine was *N*-acetylated by human hepatocytes to form *N*-acetylated metabolites. However, there was no evidence for the formation of mono-oxygenated metabolites or for enzyme-mediated covalent binding of [^{14}C]*p*-Phenylenediamine to microsomal protein. Unlike [^{14}C]*p*-Phenylenediamine, 2-aminofluorene underwent CYP mediated metabolism to 4 different hydroxylated metabolites.

Animal

Dermal

p-Phenylenediamine HCl

The metabolic profile in plasma of [^{14}C]*p*-Phenylenediamine HCl was investigated following a single occlusive dermal application at 49.9 mg/kg bw in 3 male and 3 female Sprague-Dawley rats for 4 h.[7] The solvent was 40% ethanol. The levels of radioactivity in the plasma collected at the end of the exposure period were 1412 and 7401 ng_{eq}/g for males and females, respectively. The observed radioactivity corresponded to DAPPD only.

Oral

p-Phenylenediamine

The absorption, distribution, metabolism, and excretion of *p*-Phenylenediamine was studied using male and female Fischer 344 rats and male and female B6C3F$_1$ mice.[22] The test material was dissolved in a solution of (1:1) ethanol and polyoxyethylated castor oil; water was added to yield a final solvent ratio of 1:1:8. Radiolabeled test material was diluted, as needed, with nonlabelled *p*-Phenylenediamine to administer 15 µCi/kg at each dose level. The doses administered orally

were 60 and 600 µmol/kg in 1 ml/kg of the dosing solution. Each mean value relating to the distribution or excretion of *p*-Phenylenediamine-derived radioactivity was obtained with 3 animals each at time points from 15 min to 3 d after administration.

In rats and mice (both sexes of each species), *p*-Phenylenediamine was readily absorbed, distributed to all major tissues examined, and metabolized to several metabolites (metabolites not described). These metabolites were rapidly cleared from the body mainly through the urine, and, to a lesser extent, through the feces. Absorption was described as rapid, and, in most cases, excretion in urine was more than 90% complete within the first 24 h. The cumulative recovery of radiolabeled test material in the urine was 61.5 - 73.5% in male mice, 78.3 - 87.4% in female mice, 75.7 - 81.5% in male rats, and 65.0 - 68.6% in female rats. In the feces, cumulative recovery was 15.0 - 25.1% in male mice, 18.5 - 26.1% in female mice, 13.6 - 33.4% in male rats, and 14.6 - 32.1% in female rats. Male mice had higher test material-derived radioactivity concentrations in the liver, whereas females had higher concentrations in muscle. When residual concentrations of test material-derived radioactivity in the tissues of mice and rats were compared, the values were found to be in the same range, except for the kidney and muscle. The kidney contained lower concentrations in both sexes of mice, and the muscle contained lower concentrations in male mice.[22]

p-Phenylenediamine HCl

Plasma pharmacokinetics of total radioactivity was investigated following single oral gavage administration of 6.45 mg/kg [^{14}C]*p*-Phenylenediamine to male and female Sprague-Dawley rats.[7] The plasma radioactivity versus time profiles showed a fast absorption phase (T_{max} = 0.5 h) with a C_{max} of 7.12 µg/ml for males and 6.88 µg/ml for females. A regular decrease in radioactivity levels was observed until the end of the end of the 24-h period. The respective plasma AUC_{0-t} was 24.85 $\mu g_{eq}h$/ml and 27.30 $\mu g_{eq}h$/ml for males and females, respectively. The mean recovery of administered radioactivity in 24 h for males and females, respectively, was as follows: 74.4 and 81.0% in urine, 19.3 and 13.8% in feces, 3.1 and 4.8% in cage wash, and 7.0 and 4.7% in carcasses. Total recovery was 103.8% in males and 104.4% in females.

In another 24 h study of the plasma pharmacokinetics and mass balance, total radioactivity was measured following a single oral administration of [^{14}C]*p*-Phenylenediamine HCl in water (dose = 4 mg/kg) to male and female Sprague-Dawley rats.[7] Following oral gavage, the mean plasma radioactivity levels after 0.5 h increased rapidly to C_{max} values of 4.10 µg/ml and 3.73 µg/ml for males and females, respectively. A regular decrease in radioactively levels was observed during the remaining time, with 0.015 µg/ml and 0.022 µg/ml for males and females, respectively, observed at 24 h. The mean recovery of radioactivity in the 24-h period for males and females, respectively, was as follows: 57.0 and 60.1% in urine, 23.7 and 19.3% in feces, 7.3 and 8.3% in cage wash, and 3.7 and 4.2% in carcasses. Total recovery was 91.8% in males and 92.0% in females.

In an oral study, *p*-Phenylenediamine HCl (60 mg, with 10 ml of water) was administered to male and female rabbits (number and strain not reported).[23] Following administration, *p*-Phenylenediamine and its metabolites were measured in the serum. Serum concentrations varied from 0.018 to 0.213 µg/ml for *p*-Phenylenediamine, 0.030 to 0.111 µg/ml for MAPPD, and 0.85 to 3.02 µg/ml for DAPPD. The levels of metabolites peaked during the first 0.5-h collection interval. Maximum absorption of *p*-Phenylenediamine (2.20 µg/ml) occurred at 1.5 h post-administration. Within 24 h after oral dosing, 86% of administered *p*-Phenylenediamine was found in the urine, 10% was found in the feces, and 4% was found in the blood. The major metabolite was DAPPD, and the minor metabolite was *N*-acetyl-*p*-phenylenediamine.

Othe Exposure Routes

p-Phenylenediamine

In the study of *p*-Phenylenediamine in male and female Fischer 344 rats and male and female B6C3F$_1$ mice described above, the test material was also administered intravenously at a dose of 600 µmol/kg in the tail vein.[22] The test material preparation and animal analysis were performed in a similar manner. The authors of the studies noted that excretion was not greatly affected by the route of administration.

The excretion and distribution of [^{14}C]*p*-Phenylenediamine in male rats (number and strain not specified) were determined for a 72-h period after a single 10 mg/kg intraperitoneal dose.[5] The vehicle was Tween 20 and 1.15% saline (20:80). This study was performed in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 417. Approximately 50% of the dose was excreted in the urine and 35% in the feces. Approximately 3 – 4% of the dose remained in the animal after 72 h.

Human

Dermal

p-Phenylenediamine

In a metabolism study conducted on 5 female volunteers who were long-time users of oxidative hair dyeing products, the urine (following enzymatic hydrolysis) of each subject was analyzed.[24] The *p*-Phenylenediamine content of the products habitually used by the subjects ranged from 0.54 to 2.52%. The study utilized the same formulations the subjects regularly used with unlabeled *p*-Phenylenediamine. The dying procedure was performed by a professional hairdresser according to the instructions provided by the manufacturer of the formulation. Prior to application, the subjects provided a urine sample to

serve as an analytical blank. The authors monitored the excretion of metabolites 24 or 48 h after application of the dye. Several metabolites of *p*-Phenylenediamine were hydrolyzed to free *p*-Phenylenediamine. The major metabolite that was determined using this approach was DAPPD. Approximately 80% of the *p*-Phenylenediamine recovered after flash hydrolysis was from the hydrolysis of DAPPD. The excretion of the *p*-Phenylenediamine derivatives began shortly after the dyeing procedure was terminated. Approximately 85% of the amount that could be recovered during 48 h was recovered during the first 24 h.

Urinary [^{14}C]metabolites and *N*-acetyltransferase 2 (NAT2) genotype were profiled in 8 male subjects after treatment with a dark-shade oxidative hair dye containing [^{14}C]*p*-Phenylenediamine.[25] The oxidative dye (70 ml; corresponding to a mean of 1.31 ± 0.05 g_{eq}. [^{14}C]*p*-Phenylenediamine per subject) was applied to the hair for 30 min. Application was followed by rinsing and washing with water and shampoo. The radioactivity of the formulation applied to the hair amounted to a mean total of 7.14 ± 0.26 x 10^7 dpm per subject. Urine fractions were collected from the subjects for 120 h following hair dye treatment at 4-h intervals for up to 14 h, followed by collection at 12-h intervals.

Genotyping identified 3 subjects as slow acetylators, and 5 subjects were classified as intermediate NAT2 acetylators. The subjects excreted a mean total of 0.43 ± 0.24% of the applied carbon-14 radiolabel in the urine within 24 h after treatment, and MAPPD and DAPPD (considered major urinary metabolites, present in all urine samples; accounted for 80 to 95% of the total urinary radioactivity) were two of the metabolites that were identified. Another metabolite, possibly a glucuronic acid conjugate, was identified (in 6/8 urine samples, amounting to 5 to 13% of total urinary radioactivity). All of the metabolites appeared to have been related to [^{14}C]*p*-Phenylenediamine. High molecular weight dye intermediates or corresponding metabolites were not found.

Regarding the metabolite profile for the subjects, there were no significant differences between the NAT2 intermediate and the NAT2 slow acetylator subgroups. The urine of the NAT2 slow acetylators contained MAPPD at a mean concentration of 42.2 ± 10.2% and DAPPD at a mean concentration of 54.1 ± 7.6% of the total urinary radioactivity. The corresponding mean values for the intermediate acetylators were 46.0 ± 8.9% and 45.7 ± 9.9%, respectively. The results of this study suggest that the human acetylation rate of [^{14}C]*p*-Phenylenediamine after topical application is independent of the NAT2 genotype status, most likely due to metabolism by epidermal NAT1 prior to systemic absorption.[25]

In urine samples from 5 volunteers (2 males, 3 females) who had used a commercial hair dye containing 1.1 to 1.6 g of *p*-Phenylenediamine, the major metabolite was DAPPD.[23] This metabolite was excreted in the urine for 42 h after hair dyeing. The average amount of metabolite excreted was 0.14 µg/ml per person.

In a human systemic exposure study, a group of 16 volunteers received a single application of an oxidative hair coloring product by professional hairdressers.[8] The dye applied contained on-head concentrations of [^{14}C]*p*-Phenylenediamine (1.0%), resorcinol (0.5%), and *m*-aminophenol (0.5%). The exposure time was 30 min. At the end of the exposure time, the dye was rinsed off, and the hair was shampooed, dried, and clipped. Skin tape stripping was performed on a representative area of exposed scalp surface. Urine was collected quantitatively for 48 h post-exposure, blood samples were taken at pre-test and at 2, 4, 6, 10, 24 and 48 h. A protective cap was worn for 48 h to collect residues in scalp skin scales. The urine and plasma was analyzed for *p*-Phenylenediamine, MAPPD, DAPPD, trimers, and respective potential *N*-mono- and *N*,*N*'-diacetylated metabolites of the dye trimers.

The overall mass balance obtained in this study was 96.21 ± 1.57%. The bulk of radioactivity was recovered in washing water and hair, which contained means of 64.6 and 30.2% of the applied radioactivity, respectively. Urinary excretion of [^{14}C]*p*-Phenylenediamine equivalents represented 0.88 ± 0.46% of the applied radioactivity. In all plasma samples, *p*-Phenylenediamine and MAPPD levels were below respective lower limits of quantification (< 500 pg/ml and < 1000 pg/ml, respectively), whereas significant amounts of DAPPD were found in the plasma from 2 to 48 h. Plasma kinetic mean data yielded a C_{max} of 97.4 ± 61.5 ng/ml, a T_{max} of 2 h, and an $AUC_{0-\infty}$ of 966 ± 575 ng * h/ml, respectively. Some kinetic data indicate considerable differences between individuals (up to 11-fold).

Scalp stratum corneum residues collected by skin stripping contained mainly *p*-Phenylenediamine. Only DAPPD was found in the plasma; *p*-Phenylenediamine or MAPPD were not detected (0.5 and 1.0 ng/ml lower limits of quantification, respectively). Hair dye reaction products, i.e., trimers or mono- or diacetylated metabolites of trimers, were not detected in most plasma samples. A few samples occasionally contained traces of trimers or mono- or diacetylated trimers slightly above their lower limits of quantification (0.1 – 0.32 ng/ml), suggesting negligible systemic exposure to these compounds. Urine samples mainly contained DAPPD (>99% of the substances found); some samples also contained very low levels of *p*-Phenylenediamine (mean <0.3%) or MAPPD (mean <0.2%). Hair dye reaction products were generally not detectable in urine samples. In a few samples, traces of trimers and mono-acetylated trimers were detected in the 0 – 12 h urine samples. When comparing human systemic exposure levels of reaction products with that of DAPPD, exposure to reaction products was 3 to 4 orders of magnitude lower. Some kinetic data indicate considerable differences between individuals (up to 16-fold).[8]

In a study performed in the same manner as described above, 16 volunteers received an oxidative hair dye containing radiolabeled 2% *p*-Phenylenediamine, as well as resorcinol (1.0%) and *m*-aminophenol (1.0%).[8] The overall mass balance obtained in this study was 94.30 ± 3.01%. The bulk of radioactivity was recovered in washing water and hair, which

contained a mean of 61.5 and 31.7% of the applied radioactivity, respectively. Urinary excretion represented 0.72 ± 0.25% of the applied radioactivity. Plasma kinetic mean data yielded a C_{max} of 132.6 ± 52 ng/ml, a T_{max} of 2 h, and an $AUC_{0-\infty}$ of 1415 ± 592 ng * hr/ml, respectively.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

No signs of toxicity were observed when dry p-Phenylenediamine or a 10% alcoholic solution of p-Phenylenediamine was applied to a 25 cm² area of the skin of rabbits.[2] The dermal LD_{50} for rabbits of a hair dye composite containing 1.2% p-Phenylenediamine was greater than 10 g/kg. Edema and focal necrosis were observed in rats following skin applications of 1 to 5 mg p-Phenylenediamine; similar reactions were reported for subcutaneous administration of 1 to 5% p-Phenylenediamine HCl in 0.9% sodium chloride. Acute oral toxicities of p-Phenylenediamine and p-Phenylenediamine HCl and formulations containing these ingredients have been studied in rats, rabbits, cats, and dogs. The acute oral LD_{50} of p-Phenylenediamine for rats ranged from 80 to 98 mg/kg; p-Phenylenediamine was classified as moderately toxic. The acute intraperitoneal LD_{50} of an aqueous p-Phenylenediamine solution for rats was 37 mg/kg. The subcutaneous minimum lethal doses of p-Phenylenediamine were 170 mg/kg for rats, 200 mg/kg for rabbits, and 100 mg/kg for dogs. Intraperitoneal administration of p-Phenylenediamine to rats and cats and subcutaneous administration of p-Phenylenediamine to rats, rabbits, and guinea pigs resulted in edema of the head and neck.

Acute toxicity studies on *p*-Phenylenediamine are summarized in Table 4. In dermal rabbit studies, the LD_{50} of *p*-Phenylenediamine was > 7940 mg/kg and mortalities were observed in another study at the maximum dose tested of 5000 mg/kg.[5] In oral studies, mice that received up to 70 mg/kg *p*-Phenylenediamine had a statistically significant increase in serum creatine phosphokinase (CPK) and aldolase after 24 and 72 h and rhabdomyolysis was observed after 24 h.[5] The minimal non-lethal oral dose of *p*-Phenylenediamine was 75 mg/kg in rats that received up to 100 mg/kg *p*-Phenylenediamine.[5,7] Dogs that received up to 100 mg/kg *p*-Phenylenediamine orally had marked edema of the face, extremities, and external genitals, painful muscle rigor accompanied with massive necrosis of the skeletal muscles, and increases in serum CPK and serum glutamic oxaloacetic transaminase (SGOT).[5] The calculated LC_{50} for *p*-Phenylenediamine in rats in a 4-h inhalation study was 0.92 mg/l in a study that tested the material at up to 1.8 mg/l.

Repeated Dose Toxicity Studies

Subchronic and chronic dermal administration of hair dye products containing up to 4% p-Phenylenediamine was not toxic to mice ,rats, or rabbits.[2] Chronic topical administration of 10% p-Phenylenediamine solutions was not toxic to mice or rabbits. Oral doses of 20 mg/kg/d p-Phenylenediamine for 12 to 13 d in rabbits increased blood concentrations of α-, β-, and γ-globulins and decreased serum concentrations of albumin and total protein. A decreased albumin:globulin (A:G) ratio was also observed. P-Phenylenediamine administered to rabbits daily in oral doses of 10 mg/kg for 90 d increased serum globulin concentration and total protein content and caused a decrease in the A:G ratio; no change in serum albumin concentration was noted. Dietary p-Phenylenediamine HCl at concentrations of 3160 ppm to rats and 4640 ppm to mice for 7 wk and 1250 ppm to mice and rats for 103 wk did not result in any signs of toxicity.

Repeated dose toxicity studies on *p*-Phenylenediamine are summarized in Table 5. In guinea pigs, activity of β-glucuronidase, acid phosphatase, glutathione-transferase, and glutathione peroxidase were significantly elevated, and lipid peroxidation and histamine were increased significantly in a 30-d dermal study of a 1% solution of *p*-Phenylenediamine in ethanol.[5] The lowest-observed-adverse-effect level (LOAEL) was 1 mg/kg bw/d in a dermal rat study of *p*-Phenylenediamine tested at up to 3 mg/kg bw/d. Effects observed included hemolytic anemia and increased sequestration of damaged erythrocytes within splenic sinuses. Mild erythema was the only adverse effects observed in a 4-mo dermal guinea pig study of a hair dye formulation that contained *p*-Phenylenediamine.

In oral studies, the no-observed-adverse-effect level (NOAEL) for *p*-Phenylenediamine was < 5 mg/kg bw/d in a 14-d study in rats when tested at up to 40 mg/kg/d.[9] Observed effects included increased lactate dehydrogenase and CPK levels at 5 mg/kg or greater, increased mean absolute and relative liver weights in 40 mg/kg males, and increased mean relative thyroid weights in 10 mg/kg or greater females. The no-observed-effect level (NOEL) in a 7 wk dietary study was 681 ppm for females and 1000 ppm for males exposed to up to 3160 ppm *p*-Phenylenediamine, based on decreased weight gains (no other effects were described).[5] A dose-dependent retardation of growth was observed in rats fed 0.05% - 0.4% *p*-Phenylenediamine for 12 wk.[26] Other effects noted were increased liver-to-body weight and kidney-to-body weight ratios and near total mortalities in the 0.4% dose group. In a 90-d oral study in rats, the NOEL was 4 mg/kg/d and the NOAEL was 16 mg/kg/d when *p*-Phenylenediamine was administered at up to 16 mg/kg/d.[5,9] Mean absolute and body-weight-related liver weights were significantly increased for 8 and 16 mg/kg/d males and absolute and body-weight-related kidney weights were increased for 8 and 16 mg/kg females. In a 13-wk oral neurotoxicity study in male and female rats that received up to 16 mg/kg bw/d *p*-Phenylenediamine in sterile water, the NOEL was determined to be 8 mg/kg bw/d and the NOAEL was determined to be 16 mg/kg bw/d.[9] At 16 mg/kg bw/d, increased incidence of wet chin in both sexes and in wet inguen and/or wet perineum was observed in females; these effects were considered to be pharmacological responses. Neuropathology evaluations did not reveal abnormalities within the nervous system of skeletal muscle.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

*Oral administration of 40 mg/kg p-Phenylenediamine to pregnant rats resulted in the deaths of 2 of 10 rats and decreased body weight.[2] No other signs of toxicity were observed at the 40 mg/kg dose or at a 30 mg/kg dose. Hair dyes containing 1 to 4% p-Phenylenediamine were applied to the skin of pregnant rats at a dose of 2 ml/kg/d after being mixed with an equal volume of hydrogen peroxide on gestation days 1, 4, 7, 10, 13, 16, and 19. No adverse effects on reproduction were observed, and the hair dyes were not teratogenic. A hair dye containing 3% p-Phenylenediamine was mixed with hydrogen peroxide, and 0.05 ml of the mixture was applied 2 times per week to female mice prior to mating and throughout gestation. There were no adverse effects on reproduction. The dye was not teratogenic, although there may have been a retarding effect on fetal ossification. The same hair dye containing 3% p-Phenylenediamine was applied dermally at a dose of 2.0 ml/kg two times a week to female rabbits from prior to mating through gestation. The dye was mixed with hydrogen peroxide immediately before use. There were no adverse effects on rabbit reproduction, and the dye was not teratogenic. Treated rabbits had a fetal survival rate of 85.4% while the fetal survival rate was 93.8% in the control rabbits. The surviving fetuses were of normal weight and length. Reproduction was unaffected, and teratogenicity was not observed after the dermal application of 0.5 ml of hair dyes containing 2% to 4% p-Phenylenediamine 2 times a week to 3 generations of mice. The dyes were mixed with hydrogen peroxide before use. A hair dye containing 2.20% p-Phenylenediamine was applied to the skin of male rats in a dose of 0.5 ml two times a week for **10** weeks after being mixed with an equal volume of hydrogen peroxide. The rats were mated, and their male offspring were also mated. No adverse effects on reproduction were observed.*

Developmental and reproductive toxicity studies on *p*-Phenylenediamine are summarized in Table 6. No adverse effects on reproduction or litter parameters were observed in a rat multigeneration dermal study with *p*-Phenylenediamine at up to 4% in oxidative formulation.[27] In a 90-d dermal study in male rats with up to 3 mg/kg/d *p*-Phenylenediamine in water, a statistically significant decrease in absolute testes weigh and total sperm count with abnormal testicular tissue morphology and a statistically significant increase in the percentage of abnormal sperm morphology were observed in the 2 and 3 mg/kg/d dose groups.[28] In an oral reproductive study in female mice, the meiotic capacity of oocytes and fertilization potential was affected by *p*-Phenylenediamine in dimethyl sulfoxide (DMSO) at up to 50 mg/kg.[29] The maternal NOEL was 5 mg/kg/d and the developmental NOAEL was 10 mg/kg/d in an oral developmental toxicity study of female rats that received up to 20 mg/kg/d *p*-Phenylenediamine in water on gestation days 6 through 19.[5] Dams experienced slightly transient lower mean gestation body weight gain in the 10 and 20 mg/kg/d dose groups, and an equivocal increase in the incidence of early resorptions and lower fetal weight and mean gravid uterus weight were observed in the 20 mg/kg/d dose group. The test material was considered non-embryo-fetotoxic.

GENOTOXICITY

p-Phenylenediamine, with and without hydrogen peroxide, was negative in Ames tests without metabolic activation; both positive and negative results with metabolic activation have been reported.[2] Different research groups have used different solvents, chemicals for induction, metabolic activation systems, and slight modifications to the Ames test procedure, with any or all of these potentially explaining the observed differences in results. Several oxidation products of p-Phenylenediamine were positive in the Ames test.

The urine of rats that received p-Phenylenediamine intraperitoneally 3 times/wk for 8 wk was not mutagenic in the Ames test.[2] The urine of rats that received p-Phenylenediamine-resorcinol conjugates topically was mutagenic with metabolic activation and was not mutagenic without metabolic activation. Women collected their urine before and after using hair dyes containing 0.46 – 2.55% p-Phenylenediamine; in the Ames test with metabolic activation their urine was not more mutagenic after hair dye application.

p-Phenylenediamine was not mutagenic in the rat micronucleus test after oral administration of two 500 mg/kg doses.[2] Oral administration of 200 mg/kg p-Phenylenediamine to male mice depressed testicular DNA synthesis. p-Phenylenediamine was not active at intraperitoneal doses of 5 – 20 mg/kg/d for 5 d in the mouse sperm-head abnormality test. p-Phenylenediamine was negative in a rat hepatocyte primary culture/ DNA repair test. Positive results were obtained for p-Phenylenediamine in the mouse lymphoma forward mutation assay.

In vitro genotoxicity studies on *p*-Phenylenediamine and *p*-Phenylenediamine HCl are summarized in Table 7. *P*-Phenylenediamine was mutagenic or weakly mutagenic in multiple Ames tests when tested with metabolic activation at up to 100,000 µg/plate, but these results were often not seen when tested without metabolic activation.[5,30-33] *p*-Phenylenediamine HCl at up to 6666 µg/plate was mutagenic in Ames tests (performed under oxidative conditions in a couple of studies), but had mixed results when tested with or without metabolic activation or when other components (like resorcinol) were tested in addition.[34-38] Mixed results were observed in additional bacterial strain genotoxicity studies with up 5 mg/ml *p*-Phenylenediamine.[5,39,40] In *Saccharomyces cerevisiae*, *p*-Phenylenediamine was not mutagenic in a mitotic recombination assay when tested at up to 0.1%, with and without metabolic activation, but it was mutagenic in a gene mutation assay when tested at up to 0.3 mM.[5] Assays in Chinese hamster ovary (CHO) cells indicated a dose-related increase in chromosomal aberrations when up to 87 µg/ml *p*-Phenylenediamine was tested without metabolic activity.[30,31] Genotoxicity to *p*-Phenylenediamine was also observed in a sister chromatid exchange assay in CHO cells with 0.4 mM and in a micronucleus test in Chinese hamster lung (CHL) cells without metabolic activation at up to 50 µg/ml.[5] Mutagenicity to

p-Phenylenediamine HCl was reported in forward mutation assays with L5178 mouse lymphoma cells when tested at up to 400 µg/ml with metabolic activation and at up to 10 µg/ml without metabolic activation;[41,42] however, no mutagenicity to *p*-Phenylenediamine HCl was reported in the same cell lines in a gene mutation assay at the *hprt* locus at up to 1000 µg/ml with metabolic activation and at up to 80 µg/ml without metabolic activation.[35] *p*-Phenylenediamine was not genotoxic in an unscheduled DNA synthesis assay in rat hepatocytes at up to 1 µmol/ml without metabolic activation, but increased expression of mutant p53 and COX-2 proteins was observed in a single cell gel/comet assay with SV-40 immortalized human uroepithelial cells at up to 40 µg/ml.[5] Genotoxicity was observed in a micronucleus test in human lymphocytes with *p*-Phenylenediamine HCl with metabolic activation at up to 2000 µg/ml with and at up to 125 µg/ml without metabolic activation.[35]

In vivo genotoxicity studies on *p*-Phenylenediamine and *p*-Phenylenediamine HCl are also summarized in Table 7. *P*-Phenylenediamine was not genotoxic in micronucleus tests in mice (intraperitoneal administration at up to 32.4 mg/kg) or rats (oral administration at up to 300 mg/kg),[5,7] and *p*-Phenylenediamine HCl was not genotoxic in a micronucleus test in rats (at up to 100 mg/kg).[43] No genotoxicity was reported in rats to *p*-Phenylenediamine in an unscheduled DNA synthesis assay or a comet assay at up to 100 mg/kg, in each respective test.[5,7]

DNA Binding

<u>*p*-Phenylenediamine HCl</u>

A single dose of *p*-Phenylenediamine HCl (600 µmol/kg; 500 µCi/ml/kg) was administered to male and female Fischer 344 rats and male and female $B6C3F_1$.[22] The route of exposure was not reported. The animals were killed at 16-h post-administration and livers were removed; DNA was isolated and purified. Protein from the livers was isolated, purified, hydrolyzed, and then analyzed for possible covalently bound *p*-Phenylenediamine-derived radioactivity. There was no evidence of covalent binding of *p*-Phenylenediamine or metabolites with hepatic DNA at the level of detection (1 pmol/mg DNA). Protein-bound *p*-Phenylenediamine radioactivity was observed in the livers of rats and mice (males and females, both species). The authors noted that covalent binding to protein does not necessarily imply toxicity, and that the covalently bound material may have eventually been eliminated during normal protein turnover.

<u>CARCINOGENICITY STUDIES</u>

In a bioassay performed by the National Cancer Institute (NCI), p-Phenylenediamine HCl in the feed of rats and mice at concentrations of 625 and 1250 ppm for 103 wk was not carcinogenic.[2] There was no evidence of a carcinogenic effect after the oral administration of 0.06 to 30 mg/kg/d p-Phenylenediamine for 8 mo to small numbers of rats. p-Phenylene-diamine was not carcinogenic in assays in which 5% and 10% solutions were applied topically twice a week in doses of 0.02 ml to mice for their lifetime and to female rabbits for 85 wk. Three hair dyes containing 1.5% p-Phenylenediamine were mixed with hydrogen peroxide before use, and 0.05 ml was applied topically to mice weekly or every 2 wk for 18 mo; carcinogenic activity was not observed. No evidence of a carcinogenic effect was found after the topical administration of 0.5 ml weekly for 2 yr to mice of 2 hair dyes containing 1.5% p-Phenylenediamine and mixed with hydrogen peroxide immediately before use. No carcinogenic effects were observed when 4 hair dye composite formulations containing 1 to 4% p-Phenylenediamine were mixed with hydrogen peroxide and 0.025 ml of the dyes were applied topically weekly for 21 to 23 mo to mice. Three hair dye formulations containing 2 to 4% p-Phenylenediamine were mixed with an equal volume of hydrogen peroxide and applied topically to a parental generation of rats from the time of their weaning to the weaning of their young. The second generation received topical applications of 0.5 ml two times a week for 2 yr. An increase in pituitary adenomas was observed in the rats receiving the 4% formulation; these adenomas have a high background incidence in rats.

In a discussion of the results from the NCI bioassay on p-Phenylenediamine HCl, it was noted that this dye induced an elevated incidence of bladder tumors (mainly transitional cell papillomas and carcinomas) in female rats, but that this finding was not statistically significant. Furthermore, this dye was associated with an elevated incidence of kidney tumors (tubular cell adenomas and transitional cell carcinomas of the pelvis) in male and female rats that was not statistically significant. In light of these data, the author stated that the rarity of spontaneous bladder and kidney tumors among historical control rats indicated that the low, but elevated, incidence of these tumor types may be treatment-related. It was also noted that p-Phenylenediamine HCl was associated with an elevated incidence of liver tumors in female mice that was not statistically significant, but that this finding was possibly a treatment-related tumor response. Further analysis of the NCI bioassay, it was determined to be unreasonable to conclude that p-Phenylenediamine HCl has been definitely shown to be noncarcinogenic in view of the study results and failure to achieve a maximum tolerated dose in male mice.

An expert opinion on the carcinogenic potential of p-Phenylenediamine noted that the data from animal studies do not provide any evidence of carcinogenic potential. There is very low systemic exposure to the dye or its metabolites after application of hair dye products in humans, and data show that p-Phenylenediamine cannot be converted to reactive N-hydroxyarylamine metabolites that are implicated in bladder carcinogenesis.

The International Agency for Research on Cancer (IARC), based on evaluation of data on *p*-Phenylenediamine HCl, has classified *p*-Phenylenediamine as a Group 3 chemical, not classifiable as to its carcinogenicity to humans.[44] This finding was based on no adequate human data and inadequate animal data.

Carcinogenicity and tumor promotion studies of *p*-Phenylenediamine and *p*-Phenylenediamine HCl are summarized in Table 8. No papillomas or carcinomas were reported in mice that received dermal applications of 5% *p*-Phenylenediamine twice weekly for 20 wk.[5] No tumors were observed in rats that received 20 mg/kg *p*-Phenylenediamine subdermally for 4 mo, but were observed in 2 out of 5 rats that received 12.5 mg/kg for 8 mo. A statistically significant incidence of mammary gland tumors were observed in female rats that received topical applications of 5% *p*-Phenylenediamine HCl for 18 mo.[34]

In oral studies, no carcinogenicity was observed in rats that received up to 0.1% *p*-Phenylenediamine in dietary feed for up to 80 wk,[5,26] or in mice that received 30 mg/kg *p*-Phenylenediamine HCl via gavage in a multigeneration study that lasted up to 137 wk.[5,7] No carcinogenicity was observed in mice that received *p*-Phenylenediamine or *p*-Phenylenediamine HCl intraperitoneally at up to approximately 30 mg/kg for up to 8 wk.[5,9,45] A statistically significant incidence of uterine tumors and malignant and benign soft tissue tumors were observed in female rats that received subcutaneous injections of 5% *p*-Phenylenediamine HCl for 18 mo.[34] In tumor promotion studies, *p*-Phenylenediamine did not significantly increase γ-glutamyl transpeptidase positive foci that were observed 3 wk after *N*-nitrosodiethylamine initiation in rats that received up to 1000 ppm of the test material in dietary feed, and a 40 mg/kg single dose of *p*-Phenylenediamine HCl did not cause a statistically significant increase in the number of glutathione 5-transferase positive foci when compared to controls in a 5 wk intragastric study.[46,47]

OTHER RELEVANT STUDIES

Hematological Effects

p-Phenylenediamine

In a study investigating methemoglobin formation by p-Phenylenediamine, 3.23 x 10^{-4} mol/kg of the hair dye intermediate was suspended in 0.5 ml of peanut oil and injected intraperitoneally into male rats.[2] Methemoglobin as a percentage of total hemoglobin was 3.7 ± 1.0% at 1 h, 1.4 ± 0.6% at 4 h, 3.8 ± 1.4% at 7 h, and 3.6 ± 1.5% at 10 h after injection. In vitro determinations of methemoglobin were also made. Rat erythrocytes were isolated and incubated with 10^{-3} M p-Phenylenediamine dissolved in DMSO. Methemoglobin as a percent of total hemoglobin was 2.0 ± 1.8 at 1 min, 1.2 ± 0.5 at 5 min, 1.8 ± 0.1 at 10 min, 1.8 ± 0.1 at 20 min, 2.4 ± 0.7 at 30 min, 0.5 ± 0.5 at 1 h, 3.9 ± 0.9 at 1.5 h, and 3.9 ± 0.9 at 2 h of incubation. No methemoglobin formation was observed in erythrocytes incubated with the DMSO vehicle. Additional studies demonstrated that p-aminophenol induced methemoglobin formation. The authors concluded that, when incubated together with p-aminophenol in isolated rat erythrocytes, p-Phenylenediamine had a strong inhibitory effect on methemoglobin formation.

In another study, a suspension of p-Phenylenediamine in propylene glycol was given by intraperitoneal injection to male rats at a dose of 100 µmol/kg (in a volume of 2 ml).[2] The percentage of methemoglobin formed in the blood was 12.9 ± 4.2 at 5 h after the injection. Methemoglobin formation was also studied in vitro by incubating 0.1 µmol of rat hemoglobin with 0.5 µmol of p-Phenylenediamine at 37°C for 5 h. Methemoglobin formation in vitro was 12.8 ± 0.4%, whereas the control methemoglobin concentration was 4.2 ± 1.0%.

A group of 10 pregnant rats received 40 mg/kg p-Phenylenediamine orally on days 8, 9, and 10 of gestation.[2] Two rats died after the third dose. A second group of 10 pregnant rats received 30 mg/kg p-Phenylenediamine orally on days 6 through 15 of gestation; there were 20 control rats. The animals were bled 4 to 5 h after the final dose of p-Phenylenediamine, and methemoglobin concentrations were measured as a percentage of total hemoglobin. All the control animals had methemoglobin concentrations of less than 0.1%. One rat in the 40 mg/kg group and one in the 30 mg/kg group had methemoglobin concentrations of 0.1 and 0.4%, respectively. All other treated rats had methemoglobin concentrations of less than 0.1%.

Three groups of 2 female Beagle dogs each were bled 2 d before dosing and 6 and 24 h after gastric intubation of aqueous p-Phenylenediamine solutions in doses of 1.0, 3.0, and 10.0 mg/kg.[2] Methemoglobin concentrations in the blood were measured. In an additional trial of the same experiment, 2 more female Beagle dogs received 10 mg/kg p-Phenylenediamine orally. All methemoglobin values were within the normal range.

Effects on Pigmentation

p-Phenylenediamine

p-Phenylenediamine has been observed to inhibit melanin formation in vitro.[2] The hair dye intermediate combines with ortho-quinones, which prevents the oxidation of dopa quinone to melanin. In cultures of both white and black pig skin, 10 mM p-Phenylenediamine caused "marked degeneration," a "more rapid" pyknosis, and inhibition of both arginine and tyrosine uptake into skin protein. Incorporation of tyrosine into the melanin of skin was also inhibited.

Immune Response

p-Phenylenediamine

Massive peribronchial infiltrates of eosinophils were observed in guinea pigs 72 h post-intrapulmonary administration of 1% p-Phenylenediamine solution.[2] The injected lung showed eosinophil infiltrates in response to the antigen; no eosinophilia developed in the blood, and no infiltrates of eosinophils were detected in the noninjected lung. When isolated rat

mast cells were exposed to a 0.9% saline solution with 100 µg/ml p-Phenylenediamine, it did not induce release of histamine or 5-hydroxytryptamine. p-Phenylenediamine at concentrations of 20 to 300 ng/ml had no effect on the degranulation of rat peritoneal mast cells. Histochemical staining methods revealed that Langerhans cells in isolated guinea pig and human epidermis selectively absorbed p-Phenylenediamine. [2]

Cytotoxicity

p-Phenylenediamine

Interference with mitosis was observed in intestinal cells of mice given a 0.05 mg intraperitoneal injection of p-Phenylenediamine.[2] *Glutathione depletion, lipid peroxidation and cell lysis were observed in isolated rat hepatocytes treated with 1.0 mM p-Phenylenediamine.*

The potency of *p*-Phenylenediamine in causing cytotoxic effects was studied in CHO cells.[30,31] A 50% toxic concentration (TC_{50}) of 29 ± 4 ppm was reported.

In a (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, rat skin fibroblast cells were treated with 5 – 200 µM *p*-Phenylenediamine for 24 h.[48] Cell viability was significantly ($p < 0.001$) decreased in a dose-dependent manner. The inhibitory concentration of 50% (IC_{50}) was 100 µM.

In a nonspecific cytotoxicity test of *p*-Phenylenediamine, Hartley guinea pig lymph node cells were treated with 1, 10, or 50 ppm of the test material for 96 h.[49] Notable toxicity to the lymphocytes was observed at 10 ppm and greater.

p-Phenylenediamine HCl

Cytotoxicity testing of *p*-Phenylenediamine HCl (in ≤ 0.5% acetone or ≤ 0.5% DMSO) was conducted in parallel with the testing of this dye in the C3H/10T1/2 clone 8 mouse embryo cell transformation assay at 2 different laboratories.[50] The dose ranges tested at the 2 laboratories were 0.8 to 100 µg/ml and 0.5 to 5.0 µg/ml, respectively. The methodology for determining cytotoxicity was based on the fraction of cells surviving after a 24-h treatment of 10^{-4} cells (number of cells used in transformation assay). *p*-Phenylenediamine HCl was toxic at the highest dose tested, 100 µg/ml.

Oxidative Stress

p-Phenylenediamine

Rat skin fibroblast cells were treated with *p*-Phenylenediamine (50, 100, or 150 µM) for 6, 12, or 18 h to evaluate reactive oxygen species (ROS) levels.[48] The cells were then incubated with dichloro-dihydro-fluorescein diacetate 1.6 µM) for 30 min before fluorescence intensity was measured. *p*-Phenylenediamine at all concentrations and incubation times significantly increased the level of ROS in the fibroblast cells.

Myotoxicity

p-Phenylenediamine

Rabbits that received p-Phenylenediamine at oral doses of 20 mg/kg for 12 to 13 d and 10 mg/kg for 90 d had marked alterations in myocardial parenchyma.[2] *These changes included edema, swelling of muscle fibers, cytoplasmic homogenization, and loss of cross-striation.*

The metabolic effect of *p*-Phenylenediamine in rhabdomyolysis disease was studied with *mus musculus* mouse C2C12 muscle cells (154 µM) and in groups of 3 Wistar rats (10, 20, 40, or 60 mg/kg bw in DMSO via single gavage dosing).[51] Mass isotopomer distribution analysis and computational modeling approaches were used to measure metabolic profile of C2C12 cells treated by *p*-Phenylenediamine. After 24 h treatment, *p*-Phenylenediamine induced S phase arrest, resulting in apoptosis of 40% of C2C12 cells. After incubating C2C12 cells with [1,2-^{13}C2]-glucose for 24 h and then measuring the distribution of ^{13}C isotopologues in key metabolites of glucose metabolic network, a computational fluxomic analysis showed that *p*-Phenylenediamine inhibits glycolysis, non-oxidative pentose phosphate pathway, glycogen turnover, and the ATPase reaction resulting in decreased ATP synthesis. The rats treated with 10 or 20 mg/kg *p*-Phenylenediamine showed depressed activity and myoglobinuria 10 h after treatment. After 24, 48, and 72 h, treatment with *p*-Phenylenediamine at 40 and 60 mg/kg showed an increase of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and creatine kinase. Blood packed cell volume and hemoglobin levels, as well as organs weight at 48 and 72 h, were also measured; no statistically significant differences were observed in these parameters under any condition. The authors concluded that *p*-Phenylenediamine induced some pathologic signs involved in rhabdomyolysis.

Hepatotoxicity

p-Phenylenediamine

No hepatic toxicity was observed in male rats given a single 100 µmol/kg intraperitoneal injection of p-Phenylene-diamine in propylene glycol.[2]

Neurotoxicity

p-Phenylenediamine

In an acute neurotoxicity study, groups of 24 Crl:CD rats (12 males, 12 females) were dosed by gavage with p-Phenylenediamine in sterile water at single doses of 20, 40, and 80 mg/kg.[4] The control group was dosed orally with the vehicle only. Females had significant dose-related effects on body weight gain and males had similar effects, but only at the 2 higher doses. In the functional observational battery assessments, females had statistically significant dose-related signs of general malaise. Males had similar responses, but they were not statistically significantly different from the controls. Decreased motor activity (dose-related) was demonstrated; however, in the absence of other signs of neurological impairment, the motor activity response was interpreted as being indicative of general malaise at the doses of p-Phenylenediamine that were tested.

DERMAL IRRITATION AND SENSITIZATION

Primary skin irritation by 2.5 to 100% p-Phenylenediamine varied from none to slight in experiments with rabbits, guinea pigs, mice, miniature piglets, piglets, dogs, and baboons.[2] A hair dye containing 1.2% p-Phenylenediamine produced slight to moderate erythema and moderate edema in the skin of rabbits. Another hair dye containing 1.8% p-Phenylenediamine was mildly irritating to the skin of rabbits. The primary irritation index for 50% p-Phenylenediamine applied to the skin of 6 human volunteers for 24 h under occlusive conditions was 0.8 of a maximum possible total of 8.

p-Phenylenediamine is a strong sensitizer in guinea pigs using a variety of test methods; induction routines and challenge patches with 0.001 to 10% p-Phenylenediamine sensitized 56 to 100% of guinea pigs tested.[2] However, in formulation, 2% p-Phenylenediamine was not a sensitizer in 12 guinea pigs. In a clinical study with 24 subjects, all were sensitized after five 48-h induction patches of 10% p-Phenylenediamine. The subjects had been challenged with a non-irritating concentration of p-Phenylenediamine (no further details). A maximization test using 2% p-Phenylenediamine for induction sensitized 15 of 34 (44%) male subjects. A 10% aqueous solution of a dye formulation containing 2% p-Phenylenediamine was used for nine 24-h induction patches; at challenge, significant dermatitis was observed in 7 of 22 (31.8%) of the volunteers. Human repeated insult patch tests (HRIPTs) were conducted on 206 subjects with four hair dyes containing up to 2.144% p-Phenylenediamine; the hair dyes did not cause irritation or sensitization. A p-Phenylenediamine photopatch was conducted on 1 subject; p-Phenylenediamine was not phototoxic.

Additional guinea pig studies reported sensitization to p-Phenylenediamine, with challenge concentrations as low as 0.01%.[4] Human patch testing indicated p-Phenylenediamine was sensitizing.

Dermal irritation and sensitization studies are summarized in Table 9. *p*-Phenylenediamine Sulfate was predicted to be not irritating in human reconstructed epidermis when tested neat.[6] *p*-Phenylenediamine was not irritating or mildly irritating in several guinea pig studies when tested at up to 30%.[5] In rabbit studies at up to 100%, mild irritation was observed to p-Phenylenediamine, but it was not corrosive.[5,7] *p*-Phenylenediamine was sensitizing in several local lymph node assays (LLNAs) in mice and in one LLNA using guinea pigs, with an estimated concentration of a stimulation index (SI) of 3 (EC_3) determined to be 0.06% in a study of up to 1.25% *p*-Phenylenediamine.[5,7,52] It was also sensitizing in numerous guinea pig studies when induced at concentrations of 0.1 – 1% and challenged at concentrations of up to 30%.[5,53] *p*-Phenylenediamine was sensitizing in predictive studies in human subjects when tested at up to 1% in pet.[54]

Cross-Sensitization

Animal

p-Phenylenediamine

In a sensitization study, guinea pigs were sensitized to 0.05% p-Phenylenediamine in pet.[2] Through cross-reaction, 95 to 100% of the treated guinea pigs were also sensitized to N-phenyl-N'-cyclohexyl-p-phenylenediamine (CPPD; 0.5% pet.), N-dimethyl-3-butyl-N'-phenyl-p-phenylenediamine (0.5% pet.), and N-isopropyl-N'-phenyl-p-phenylenediamine (IPPD).

The cross-sensitization potential of *p*-Phenylenediamine was studied in female Hartley guinea pigs in a modified lymphocyte transformation test.[49] The guinea pigs were divided into experimental (n =9) and control (n=5) groups and treated with *p*-Phenylenediamine or distilled water via a maximization test procedure. On day 1, the animals received injections of 0.1 ml of 0.1% *p*-Phenylenediamine in distilled water, 0.1 ml Freund's complete adjuvant, and 0.1 ml *p*-Phenylenediamine emulsified in the adjuvant at 0.1%. After 7 d, 0.5 ml of 5% *p*-Phenylenediamine in distilled water was applied with a 2 x 4 cm^2 occluded patch to the injection sites for 48 h. The control group did not receive the test material in this procedure. After 21 d, the experimental and control groups were challenged with 0.025 ml of 1% *p*-Phenylenediamine, 1% *p*-aminophenol, and 5% *m*-phenylenediamine in distilled water using Finn chambers for 24 h. Sensitization was assessed 24 h after the patches were removed. After dermal testing, the animals were killed and the lymph node cells were harvested from peripheral lymph nodes. The cells were incubated with *p*-Phenylenediamine (0, 1, or 5 ppm), *p*-aminophenol (0, 1, or 5 ppm), or *m*-phenylenediamine (0, 5, or 25 ppm), with and without suspensions of epidermal cells, prior to treatment with ^{3}H-thymidine for 24 h (total culture initiation was 120 h). The positive control for lymphocyte initiation was phytohemagglutinin. The level of ^{3}H-thymidine incorporation was determined in a liquid scintillation counter.

In the in vivo challenge, all the guinea pigs sensitized to *p*-Phenylenediamine reacted positively when challenged with *p*-Phenylenediamine. The pigmentation of *p*-aminophenol and *m*-phenylenediamine interfered with erythema determination, thus reactions could not be read. The control animals did not react. In the in vitro challenge, a statistically significant increase ($p < 0.01$) in blastogenesis in the lymph node cells from *p*-Phenylenediamine-sensitized animals was observed in a dose-dependent manner when *p*-Phenylenediamine was added to the cultures without epidermal cells. Blastogenesis from *p*-Phenylenediamine-sensitized animals was also increased in a statistically significant ($p < 0.01$) dose-dependent manner when *p*-aminophenol or *m*-phenylenediamine was added to the cultures without epidermal cells, with the response to *p*-aminophenol being greater than *m*-phenylenediamine. These responses were not observed in the control lymphocytes. The amount of blastogenesis stimulated by *p*-Phenylenediamine, *p*-aminophenol, or *m*-phenylenediamine was a little higher in the presence of epidermal cells, but the difference was not statistically significant. The authors concluded that there is cross-sensitization between *p*-Phenylenediamine and *p*-aminophenol or *m*-phenylenediamine.[49]

OCULAR IRRITATION STUDIES

Animal

p-Phenylenediamine

Mild conjunctival inflammation that did not persist for more than 24 h was observed after the instillation of a 2.5% aqueous p-Phenylenediamine solution into rabbit eyes.[2] In another study, the maximum irritation score was 17.0 out of a possible 110 after 100% p-Phenylenediamine was placed in rabbit eyes. A hair dye composite formulation containing 1.2% p-Phenylenediamine and one containing 1.8% p-Phenylenediamine were instilled into the conjunctival sacs of the eyes of rabbits producing, at 1-d post-instillation, a score of 33.0 for unwashed eyes and 23.0 for washed eyes for the low concentration and a score of 30 for unwashed eyes at the higher concentration; irritation was minimal after 7 d.

Ocular irritation studies are summarized in Table 10. Keratitis and corneal opacities were observed in rats that received up to 15% *p*-Phenylenediamine in formulation daily for up to 3 mo.[5] No ocular irritation was observed in guinea pigs with 2.5% *p*-Phenylenediamine. In rabbits, *p*-Phenylenediamine was moderately irritating when tested neat and was weakly irritating at 2.5 to 5%.[5,7]

CLINICAL STUDIES

A variety of patch tests with p-Phenylenediamine have been performed on subjects from a variety of populations.[2] Many of these reports are of diagnostic patch tests performed on clinical subjects suffering from skin disease with varying degrees of positivity rates. Case reports of dermal allergy included hair dye consumers and beauticians. Edema of the eyelids, conjunctivitis, and tearing, in addition to more severe reactions to the eyes, have been observed after application of p-Phenylenediamine hair dyes to the eyebrows and eyelashes.

A literature review of 13 relevant articles on allergic contact dermatitis and irritant contact dermatitis was performed on material published from 1980 to January 2022, with focus on skin of color patients.[55] One of the most common allergens associated with positive patch tests that was different in a statistically significant manner ($p < 0.05$) between skin of color patients (primarily Black and Indian) and white patients was *p*-Phenylenediamine.

A retrospective data analysis study to determine risk factors for *p*-Phenylenediamine sensitization was performed by the Information Network of Departments of Dermatology (IVDK) of Germany from 2008 to 2013.[56] Of 271 positive patients (n = 4314; 6.3%) surveyed, hair dyeing (odds ratio (OR) 6.0; 95% confidence interval (CI) 3.9 – 9.4), henna tattoos (OR 2.4; 95% CI 1.5-3.7), and occupation as a hairdresser (OR 2.1; 95% CI 1.3-3.2) increased the risk of *p*-Phenylenediamine sensitization, but too few patients performed the hair dye pretests to perform a detailed analysis to determine the risk from this route of exposure. *p*-Phenylenediamine sensitization acquired via henna tattoos was more intense and resulted in stronger patch test reactions and more concomitant reactions to chemically related compounds.

Clinical Reports

In a study of patients with known sensitivity to *p*-Phenylenediamine, 15 patients received serial dilutions of 1% *p*-Phenylenediamine in pet. (1 – 10,000 ppm) on the upper back with Finn chamber for 48 h.[57] An additional 3 concentrations (50, 100, and 500 ppm) were also applied to the retroauricular area and lateral aspects of the upper arms. Fourteen out of the 15 patients reacted to 1 or more of the test samples. The threshold value for 10% of tested persons (ED_{10}) was 38 ppm. There were no statistically significant differences in sensitivity of the 3 regions tested.

In another provocative test, a group of 7 patients with a known sensitivity to *p*-Phenylenediamine was tested with 1% *p*-Phenylenediamine in pet. for 15, 30, and 120 min.[58] Another 9 patients with sensitivity were tested with 0.01 – 1% *p*-Phenylenediamine for 15, 30, and 120 min. The patches were 8 mm Finn chambers. At 120 min, 11/16 patients reacted to 1% *p*-Phenylenediamine and 2/9 reacted to 0.01%. At 15 min, 6/16 reacted to 1% and 0/9 reacted to 0.01%. Most of the reactions were 1+ and 2+.

In a study of patients with severe allergic reactions to permanent hair dyes, 2 patients were tested with 1% *p*-Phenylenediamine in pet., 2 patients were tested with titrated *p*-Phenylenediamine in pet. at 0.01 to 1%, and the remaining 5 patients were tested with < 1% of the test material.[59] The patches were applied with Finn chambers on back skin under

occlusion for 48 h and reactions were read on days 2 and 3. Eight out of 9 patients responded strongly to the test material. In the initial 2 patients with 1% *p*-Phenylenediamine, severe bullous reactions occurred. Severe reactions were observed with 0.1 and 0.5% *p*-Phenylenediamine, but 50% of patients did not react to the test material at 0.01%.

Multicenter and Retrospective Studies

The results of numerous multicenter and retrospective studies over the last 40 years are summarized in Table 11. Sensitization to *p*-Phenylenediamine has been observed around the globe, with sensitization rates varying greatly, independent of region or span of time.[60-95] Using the US as an example, a retrospective study at the Mayo Clinic from 2001 to 2005 of patients with suspected allergic contact dermatitis reported a positivity rate of 4.5% to *p*-Phenylenediamine, while the rate reported from 2006 to 2010 was reported as 5.2%.[77] In other countries in patients with suspected allergic contact dermatitis, Greece reported a positivity rate as high as 52.5% (2010 to 2019) and India has reported a rate of 67.5% (dates not reported).[86,89] In contrast, a German multicenter study reported a positivity rate of only 1.5% in 1994 to 1995.[66]

Case Reports with Hair Dye Products

Numerous cases of adverse reactions to hair dye products containing *p*-Phenylenediamine have been reported in the published literature, and several are summarized in Table 12.[96-105] In addition to reports of dermal reactions that have been summarized, case and cohort studies of acute *p*-Phenylenediamine intoxication through accidental or intentional oral ingestion of the dye have been reported; please note, these case reports are not included in the table because they are not relevant to the cosmetic use of *p*-Phenylenediamine.[106-114]

Case Reports Related to Temporary Tattooing

Numerous case reports of reactions following application of dark (black) henna tattoos/temporary tattoos containing *p*-Phenylenediamine are summarized in Table 13.[115-133] Most of these cases have occurred outside of the US. The US FDA has determined that uses of *p*-Phenylenediamine other than as a hair dye are unapproved, including use in dark (black) henna tattoo products.[134] In 2001, the FDA established a reporting hotline prompted by an escalation of reported severe allergic reactions to *p*-Phenylenediamine-adulterated temporary tattoos. Over a 12-yr period between 2002 and 2014, a total of 70 cases of adverse reactions to temporary tattoos (n = 6) and black henna tattoos (n = 64) were reported in the US. In 2008, the American Academy of Dermatology endorsed a ban on the practice of applying *p*-Phenylenediamine-adulterated henna tattoos due to the potential for allergic contact dermatitis to result from application.[135]

Case Reports of Skin Depigmentation

In a case report, a 50-yr-old female presented with depigmentation on both feet, corresponding to sites where alta (scarlet-red solution used by some Indian women as a cosmetic to color feet) had been applied.[138] Itching and scaling were also reported. Patch testing (48-h patch) with 1% *p*-Phenylenediamine (in plastibase) resulted in a + reaction and depigmentation at the application site.

In another case report, a 55-yr-old female applied a hair dye containing 16% *p*-Phenylenediamine for 3 h.[139] Itching and burning over the entire scalp were reported after 8 to 10 applications of this type. Within 24 h, exudation and edema over the scalp, which subsided with time, were observed. Depigmentation of the scalp was noted 3 to 4 wk later. At 1.5 yr after the onset of depigmentation (hair dye not used for 1.5 yr), depigmentation was noted over the scalp, back of neck, and forehead.

A 50-yr-old male presented with severe itching and depigmentation of beard area and temporal region of scalp that began approximately 10 mo prior.[140] The patient connected the reaction to hair dye he had been using for 2 yr. Patch testing with a hairdressing series yielded a positive reaction to *p*-Phenylenediamine and 4-aminophenol.

Case Reports of Other Cutaneous Reactions

In 50 patients with lichen planus pigmentosus and Fitzpatrick skin types IV and V, closed patch tests with the Indian standard series and the patients' cosmetic products were performed.[141] Photo-patch testing was also performed with the Scandinavian photo-patch series. A total of 28 patch tests were positive, with *p*-Phenylenediamine positive in 5 patients. Four of the patients also tested positive to commercial hair dyes, with 3 of these patients also testing positive to nickel sulfate, colophony, and neomycin sulfate. *p*-Phenylenediamine did not cause any positive reactions in the photo-patch test.

Clinical Reports of Cross-Sensitization

Allergic sensitivity to p-Phenylenediamine has been associated with cross sensitization to numerous other chemicals.[2] These chemicals include azo and aniline dyes; procaine; benzocaine; p-aminobenzoic acid and its esters; N-isopropyl-N'-phenyl-1,4-phenylenediamine; CPPD; p-aminosalicylic acid; hydrodiuril; carbutamide; pyrogallol; sulfonamides; hydroquinone; hydrochlorothiazide; p-hydroxybenzoic acid esters; benzidine; phenylhydrazine; and p-toluenediamine.

In a retrospective study of *p*-Phenylenediamine sensitization by the North American Contact Dermatitis Group (NACDG), the most common co-reactions were benzocaine (11.3%, 349/3095), IPPD (6.7%, 33/493), disperse dyes (6.5%, 159/2459), and black rubber mix (5.1%, 126/2459).[69]

The rate of cross-reactivity between parabens, *p*-Phenylenediamine, and benzocaine was evaluated in a population of patients patch-tested in a hospital-based dermatitis clinic.[143] A retrospective analysis of 4368 patients with eczematous skin disease consecutively patch-tested between July of 1989 and June of 2005 was conducted. The test materials were placed on the patient's upper back and remained for 2 d. Reactions were scored after 48 and 96 h according to ICDRG guidelines. The

positive reactions in the group of 4368 patients were reported as follows: 253 (5.7%) to *p*-Phenylenediamine, 37 (0.8%) to benzocaine, and 34 (0.7%) to the paraben mix. Of the 253 patients with positive patch test reactions to *p*-Phenylenediamine, 23 (9%) also had positive reactions to benzocaine and 6 (2.37%) had positive reactions to parabens. The results of this study indicated that the rate of cross-reactions to parabens in *p*-Phenylenediamine- and benzocaine-positive patients combined was 2.0%. The authors concluded that this cross-reaction rate is significant in the tested population, but still falls within the previously reported rates of sensitivity to parabens in the general population (0 to 3.5%).

A retrospective analysis of clinical data collected in a contact allergy surveillance network (IVDK) between January of 1992 and June of 2004 was performed to determine whether aniline should be regarded as a potential cause of contact allergy.[144] During this period, 25 of 1119 patients patch tested with aniline (1% in water or petrolatum) had positive (allergic) reactions. Of the 25 patients, 24 were diagnosed with contact allergy to *p*-Phenylenediamine (1% in pet.) and/or *p*-aminoazobenzene or another *para*-amino compound. The researchers found it unlikely that aniline was an independent sensitizer, but it may elicit allergic reactions in subjects pre-sensitized to *para*-substituted amino compounds.

A retrospective study of patients with suspected hair dye allergy in the United Kingdom between 1997 and 2007 found 68 out of 175 patients positive to *p*-Phenylenediamine, 48 positive to *p*-toluenediamine, 10 positive to resorcinol, and 13 positive to pyrogallol.[145] In this group of patients, 80 had been tested with 2-nitro-*p*-phenylenediamine, 3-aminophenol, 4-aminophenol, and 1,4-hydroquinone, which yielded 14, 9, 13, and 1 positive reactions, respectively. Of the 108 reactions to hair dye ingredients other than *p*-Phenylenediamine, only 18 occurred in the absence of a reaction to *p*-Phenylenediamine.

In 221 patients with allergic reactions to *p*-Phenylenediamine reported between 2007 and 2012 in London, 16.6% (n = 33) exhibited cross-reactions with one or more related allergens in the European baseline series.[146] Of the patients allergic to *p*-Phenylenediamine, 5.1% reacted to Disperse Yellow 3, 8.1% reacted to IPPD, and 5.6% reacted to caine mix. Cross-reactions were observed in 16% with a grade of 1+, 14.5% with a grade of 2+, 28.6% with a grade of 3+, based on the ICDRG criteria, when *p*-Phenylenediamine was tested 1% pet. When tested at 0.01 to 0.001% *p*-Phenylenediamine, cross-reactions were observed in 50% of patients with *p*-Phenylenediamine allergy.

Patch test results of patients (n = 1319) between November 2008 and June 2013 in a Vancouver Patch Test Clinic found 95 patients were positive to *p*-Phenylenediamine.[147] Of those 95 patients, 74 (78%) had at least 1 other positive reaction, with the most common co- or cross-reactants reported as nickel (31%), ammonium persulfate (23%), cobalt (II) chloride hexahydrate (20%), *p*-toluenediamine sulfate (19%), 4-aminophenol (18%), fragrance mix 1 (15%), toluenediamine base (12%), fragrance mix 2 (9%), myroxylon pereirae resin (9%), and glyceryl thioglycolate (9%).

In a prospective patch test study in 20 patients with known sensitivity to *p*-Phenylenediamine and in 19 controls, 11 patients (55%) were positive to more than one allergen in the hairdressing series (only 2 control subjects were positive).[148] Reactions were observed to *p*-toluenediamine sulfate (15%), 3-aminophenol (10%) and nickel-sulfate hexahydrate (10%).

In a retrospective study of patients with suspected allergic contact dermatitis to hair dyes from 2010 to 2019 in Greece, 199 (55%) out of 362 patients were positive to at least *p*-Phenylenediamine, *p*-toluenediamine, or *p*-aminophenol, and 163 (45%) were negative to all 3 allergens.[89] Further, 45 (12% of total population) patients were positive to both *p*-Phenylenediamine and *p*-toluenediamine, 5 (1%) to both *p*-Phenylenediamine and *p*-aminophenol, and 1 (0.003%) to both *p*-toluenediamine and *p*-aminophenol. Positive reactions to all 3 allergens were found in 86 (24%) patients. With non-hair dye allergens, all 24 patients positive to caine mix were also positive to *p*-Phenylenediamine, 4 out of 11 patients positive to methylchloroisothiazolinone/methylisothiazolinone were also positive to *p*-Phenylenediamine, and 23 out of 28 patients positive to black rubber mix were also positive to *p*-Phenylenediamine.

Occupational Exposure

The results of occupational exposure studies, mainly involving hairdressers, are summarized in Table 14.[69,92,149-155] The Occupational Safety and Health Administration (OSHA) lists the permissible exposure limit (PEL) for 8-h work shifts for *p*-Phenylenediamine as 0.1 mg/m^3.[156,157] The National Institute for Occupational Safety and Health (NIOSH) lists the recommended exposure limit (REL) for up to 10 h time-weighted average (TWA) for *p*-Phenylenediamine as 0.1 mg/m^3.

MARGIN OF SAFETY

The SCCS calculated conventional and toxicokinetic-based margin of safety values for *p*-Phenylenediamine.[8] In the conventional calculation, the margin of safety for 2% *p*-Phenylenediamine under oxidative conditions was determined to be 200. (The maximum use concentration reported to the Panel is 0.98% - 3% in hair dyes, with a maximum on-head concentration after dilution of 1%).[13] This calculation is based on the NOAEL of 8 mg/kg bw/d (from a 90-d oral rat study in which the SCCS determined the original NOAEL to be a NOEL) and a systemic exposure dose (SED) of 0.04 mg/kg bw (skin area surface of 580 cm^2 x absorption through skin of 4.47 µg/cm^2 x 0.001 (unit conversion)/typical human bw of 60 kg).

In the toxicokinetic-based approach, the margin of safety was calculated to be 23.3. This calculation used $AUC_{0-\infty}$ values from rat and human plasma concentration as systemic exposure doses. These values (for rats, 33,038 equivalents (ng-eq/g)*h, and for humans, 1415 (ng-eq/g)*h) were based on data from a 6.45 mg/kg bw kinetic rat study and from 2% on-head application of *p*-Phenylenediamine in humans, respectively. The NOAEL of 8 mg/kg bw/d was also utilized. While these results are below the threshold of 25 for toxicokinetic based margins of safety, the SCCS found the calculated value to be

borderline and had no concern regarding systemic toxicity due to the intermittent exposure to *p*-Phenylenediamine in oxidative hair dyes and the fact that human systemic exposure through hair dyeing is mainly to the de-toxified metabolite, DAPPD.

HAIR DYE EPIDEMIOLOGY

Hair dyes may be broadly grouped into oxidative (permanent) and direct (temporary or semi-permanent) dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes consist of preformed colors. *p*-Phenylenediamine and its salts are reported to be used in oxidative hair dye formulations. While the safety of individual hair dye ingredients is not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer. A detailed summary of the available hair dye epidemiology data is available at https://www.cir-safety.org/cir-findings.

SUMMARY

p-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate are reported to function as hair colorants in cosmetic products, according to the *Dictionary*. These ingredients act as primary intermediates or precursors in oxidative (permanent) hair dyes. The Panel first reviewed the safety of *p*-Phenylenediamine individually, in a report published in 1985 with the conclusion *"p*-Phenylenediamine is a known sensitizer and that some persons may be sensitized under intended conditions of use. For those persons not sensitized, the Panel concludes that *p*-Phenylenediamine is safe as a hair dye ingredient at the current concentration of use." This conclusion was reaffirmed in a re-review that was published in 2006.

Subsequently, the *p*-Phenylenediamine report was reopened to add *p*-Phenylenediamine HCl and *p*-Phenylenediamine Sulfate. The amended report was finalized in 2007 with the conclusion that these ingredients are safe as hair dyes in the practices of use and concentration as described in the safety assessment.

According to the 2023 VCRP survey data, *p*-Phenylenediamine is reported to be used in 200 formulations. The majority of these uses are in hair coloring preparations; however, 7 uses have been reported for eye makeup preparations. Only 1 use was reported in a hair coloring shampoo for *p*-Phenylenediamine HCl and no uses were reported for the sulfate salt. The frequencies of use for *p*-Phenylenediamine have greatly decreased since the initial amended report was finalized; in 2007, *p*-Phenylenediamine was reported to have 1497 uses, all in hair coloring formulations. No uses were reported at that time for the related salts.

The results of the concentration of use survey conducted by the Council in 2022 indicate *p*-Phenylenediamine has a maximum concentration of use range of 0.98% - 3% in hair dyes, with a maximum on-head concentration after dilution of 1%. No concentrations of use were reported for related salts. In the 2007 amended report, the maximum concentration of uses range for *p*-Phenylenediamine was 2% - 4% in hair dyes; the hydrochloride salt and the sulfate salt were each reported to be used at 6% in hair dyes.

Under European regulations for cosmetic ingredients, *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate are listed in Annex III with the restrictions that these ingredients may be used at only up to 6% (free base) in oxidizing hair dyes, these ingredients should not be used on eyebrows, and these ingredients are for professional use only. Annex III has been amended with the restriction that *p*-Phenylenediamine and its hydrochloride and sulfate salts may be used in products intended for coloring eyelashes when after mixing under oxidative conditions and the maximum concentration applied to eyelashes must not exceed 2% (free base); application is for professional use only.

The SCCS expressed no concern regarding systemic toxicity to use of *p*-Phenylenediamine in oxidative hair dyes at on-head concentrations of up to 2%. Further, the SCCS could not conclude on the carcinogenicity of *p*-Phenylenediamine, but decided it was unlikely that *p*-Phenylenediamine as used in hair dyes would pose a carcinogenic risk for consumers, based on toxicokinetic and genotoxicity data.

The in vitro percutaneous absorption of radiolabeled *p*-Phenylenediamine in human skin under 5 different dosing conditions was between 0.1 and 0.2% of the applied dose (1.9 - 2.4 $\mu g/cm^2$ for the complete dye formulations). For *p*-Phenylenediamine HCl, the total absorbed amount of radiolabeled was 2.4% (10.6 $\mu g_{eq}/cm^2$) in an in vitro study with human skin. An in vivo human study of 8 male subjects determined the absorbed amount of radiolabeled *p*-Phenylenediamine HCl in an oxidative hair dye to be 7.0 mg_{eq}/cm^2, with 0.5% of the applied dose found in the urine, 0.04% found in the feces, and 95.16% recovered in the washing water, cut hair, application materials, and scalp.

In human skin samples, *p*-Phenylenediamine is acetylated to MAPPD, which is then acetylated to DAPPD. In oral rat studies, *p*-Phenylenediamine and *p*-Phenylenediamine HCl were readily absorbed, distributed to major tissues, and metabolized into several metabolites that were rapidly cleared from the body, mainly in urine. The major metabolite detected in rabbits following oral administration of *p*-Phenylenediamine HCl was DAPPD. Within 24 h of dosing in the rabbits, 86% of the administered test material was found in the urine, 10% was found in the feces, and 4% was found in the blood. In

human subjects, radiolabeled *p*-Phenylenediamine in hair dye formulations applied topically was found in the urine as the metabolites MAPPD and DAPPD.

In rabbit acute dermal studies, the LD_{50} of *p*-Phenylenediamine was > 7940 mg/kg and mortalities were observed in another study at the maximum dose tested of 5000 mg/kg. In oral studies, mice that received up to 70 mg/kg *p*-Phenylenediamine had a significant increase in serum CPK and aldolase after 24 and 72 h and rhabdomyolysis was observed after 24 h. The minimal and maximum non-lethal oral doses of *p*-Phenylenediamine were 75 mg/kg and 50 mg/kg, respectively, in rats that received up to 100 mg/kg *p*-Phenylenediamine. Dogs that received up to 100 mg/kg *p*-Phenylenediamine orally had marked edema of the face, extremities, and external genitals, painful muscle rigor accompanied with massive necrosis of the skeletal muscles, and increases in serum CPK and SGOT. The calculated LC_{50} for *p*-Phenylenediamine in rats was 0.92 mg/l in a study that tested the material at up to 1.8 mg/l.

In guinea pigs, activity of β-glucuronidase, acid phosphatase, glutathione-transferase, and glutathione peroxidase were significantly elevated, and lipid peroxidation and histamine were increased significantly in a 30-d dermal study of a 1% solution of *p*-Phenylenediamine in ethanol. The LOAEL was 1 mg/kg bw/d in a dermal rat study of *p*-Phenylenediamine tested at up to 3 mg/kg bw/d. Effects observed included hemolytic anemia and increased sequestration of damaged erythrocytes within splenic sinuses. Mild erythema was the only adverse effects observed in a 4-mo dermal study of a hair dye formulation that contained *p*-Phenylenediamine.

In oral studies, the NOAEL for *p*-Phenylenediamine was < 5 mg/kg bw/d in a 14-d study in rats when tested at up to 40 mg/kg/d. Observed effects included increased lactate dehydrogenase and CPK levels at 5 mg/kg or greater, increased mean absolute and relative liver weights in 40 mg/kg males, and increased mean relative thyroid weights in 10 mg/kg or greater females. The NOEL in a 7-wk dietary study was 681 ppm for females and 1000 ppm for males exposed to up to 3160 ppm *p*-Phenylenediamine, based on decreased weight gains (no other effects were described). A dose-dependent retardation of growth was observed in rats fed 0.05 - 0.4% *p*-Phenylenediamine for 12 wk. Other effects noted were increased liver-to-body weight and kidney-to-body weight ratios and near total mortalities in the 0.4% dose group. In a 90-d oral study in rats, the NOEL was 4 mg/kg/d and the NOAEL was 16 mg/kg/d when *p*-Phenylenediamine was administered at up to 16 mg/kg/d. Mean absolute and body weight-related liver weights were significantly increased for 8 and 16 mg/kg/d males and absolute and body-weight-related kidney weights were increased for 8 and 16 mg/kg females.

No adverse effects on reproduction or litter parameters were observed in a rat multigeneration dermal study with *p*-Phenylenediamine at up to 4% in oxidative formulation. In a 90-d dermal study in male rats with up to 3 mg/kg/d *p*-Phenylenediamine in water, significant decreases in absolute testes weigh and total sperm count with abnormal testicular tissue morphology, and a significant increase in the percentage of abnormal sperm morphology were observed in the 2 and 3 mg/kg/d dose groups. The maternal NOEL was 5 mg/kg/d and the developmental NOAEL was 10 mg/kg/d in an oral developmental toxicity study of female rats that received up to 20 mg/kg/d *p*-Phenylenediamine in water on gestation days 6 through 19. Dams experienced slightly transient lower mean gestation body weight gain in the 10 and 20 mg/kg/d dose groups, and an equivocal increase in the incidence of early resorptions and lower fetal weight and mean gravid uterus weight were observed in the 20 mg/kg/d dose group. The test material was considered non-embryo-fetotoxic. In an oral reproductive study in female mice, the meiotic capacity of oocytes and fertilization potential was affected by *p*-Phenylenediamine in DMSO at up to 50 mg/kg.

p-Phenylenediamine was mutagenic or weakly mutagenic in multiple Ames tests when tested with metabolic activation and at as much as 100,000 µg/plate, but these results were not always repeated when tested without metabolic activation. *p*-Phenylenediamine HCl at up to 6666 µg/plate was mutagenic in Ames tests when tested under oxidative conditions in a couple of studies but had mixed results when tested with or without metabolic activation or when other components (like resorcinol) were tested in addition. Mixed results were observed in additional bacterial strain genotoxicity studies with up 5 mg/ml *p*-Phenylenediamine. In *S. cerevisiae*, *p*-Phenylenediamine was not mutagenic in a mitotic recombination when tested at up to 0.1%, with and without metabolic activation, but it was mutagenic in a gene mutation assay when tested at up to 0.3 mM. Assays in CHO cells indicated a dose-related in chromosomal aberrations when up to 87 µg/ml *p*-Phenylenediamine was tested without metabolic activity. Genotoxicity to *p*-Phenylenediamine was also observed in a sister chromatid exchange assay in CHO cells with 0.4 mM and in a micronucleus test in CHL cells without metabolic activation at up to 50 µg/ml. Mutagenicity to *p*-Phenylenediamine HCl was reported in forward mutation assays with L5178 mouse lymphoma cells when tested at up to 400 µg/ml with metabolic activation and at up to 10 µg/ml without metabolic activation; however, no mutagenicity to *p*-Phenylenediamine HCl was reported in the same cell lines in a gene mutation assay at the *hprt* locus at up to 1000 µg/ml with metabolic activation and at up to 80 µg/ml without metabolic activation. *p*-Phenylenediamine was not genotoxic in an unscheduled DNA synthesis assay in rat hepatocytes at up to 1µmol/ml, but increased expression of mutant p53 and COX-2 proteins was observed in a single cell gel/comet assay with SV-40 immortalized human uroepithelial cells at up to 40 µg/ml. Genotoxicity was observed in a micronucleus test in human lymphocytes with *p*-Phenylenediamine HCl with metabolic activation in 1600 µg/ml in one test, and with (up to 2000 µg/ml) and without (p to 125 µg/ml) metabolic activation in another test.

In in vivo genotoxicity studies, *p*-Phenylenediamine was not genotoxic in micronucleus tests in mice (at up to 32.4 mg/kg) or rats (at up to 300 mg/kg), and *p*-Phenylenediamine HCl was not genotoxic in a micronucleus test in rats (at up to

100 mg/kg). No genotoxicity was reported in rats to *p*-Phenylenediamine in an unscheduled DNA synthesis assay or a comet assay at up to 100 mg/kg, in each respective test. There was no evidence of covalent binding of *p*-Phenylenediamine or its metabolites with hepatic DNA when rats received a single 600 µmol/kg dose (route of exposure not reported).

IARC, based on evaluation of data on *p*-Phenylenediamine HCl, has classified *p*-Phenylenediamine as a Group 3 chemical, not classifiable as to its carcinogenicity to humans. This finding was based on no adequate human data and inadequate animal data.

No papillomas or carcinomas were reported in mice that received dermal applications of 5% *p*-Phenylenediamine twice weekly for 20 wk. No tumors were observed in rats that received 20 mg/kg *p*-Phenylenediamine subdermally for 4 mo, but were observed in 2 out of 5 rats that received 12.5 mg/kg for 8 mo. No treatment related gross lesions were observed in rats that received up to 4% *p*-Phenylenediamine in oxidative formulation dermally in a multigeneration study that lasted 24 mo. A statistically significant incidence of mammary gland tumors were observed in female rats that received topical applications of 5% *p*-Phenylenediamine HCl for 18 mo. In oral studies, no carcinogenicity was observed in rats that received up to 0.1% *p*-Phenylenediamine in dietary feed for up to 80 wk, or in mice that received 30 mg/kg *p*-Phenylenediamine HCl via gavage in a multigeneration study that lasted up to 137 wk. No carcinogenicity was observed in mice that received *p*-Phenylenediamine or *p*-Phenylenediamine HCl intraperitoneally at up to approximately 30 mg/kg for up to 8 wk. A statistically significant incidence of uterine tumors and malignant and benign soft tissue tumors were observed in female rats that received subcutaneous injections of 5% *p*-Phenylenediamine HCl for 18 mo. In tumor promotion studies, *p*-Phenylenediamine did not significantly increase γ-glutamyl transpeptidase positive foci that were observed 3 wk after *N*-nitrosodiethylamine initiation in rats that received up to 1000 ppm of the test material in dietary feed, and a 40 mg/kg single dose of *p*-Phenylenediamine HCl did not cause a statistically significant increase in the number of glutathione 5-transferase positive foci when compared to controls in a 5-wk intragastric study.

Cytotoxic effects have been reported for *p*-Phenylenediamine in CHO cells, rat skin fibroblast, and guinea pig lymph node cells. *p*-Phenylenediamine HCl was cytotoxic to mouse embryo cells at 100 µg/ml. *p*-Phenylenediamine increased the level of ROS in rat skin fibroblast cells. In mouse muscle cells and in rats in vivo, *p*-Phenylenediamine at up to 60 mg/kg in a single gavage dose induced pathologic signs involved with rhabdomyolysis. The NOEL was 8 mg/kg bw/d and the NOAEL was 16 mg/kg bw/d in a 13-wk oral neurotoxicity study of rats that received *p*-Phenylenediamine at up to 16 mg/kg bw/d.

p-Phenylenediamine Sulfate was predicted to be not irritating in human reconstructed epidermis when tested neat. *p*-Phenylenediamine was not irritating or mildly irritating in several guinea pig studies when tested at up to 30%. In rabbit studies, mild irritation was observed to *p*-Phenylenediamine, but it was not corrosive. *p*-Phenylenediamine was sensitizing in numerous guinea pig studies when induced at concentrations of up to 1% and challenged at concentrations of up to 30%. It was also sensitizing in several LLNAs in mice and guinea pigs, with an EC_3 value determined to be 0.06% in a study of up to 1.25% *p*-Phenylenediamine. *p*-Phenylenediamine was sensitizing in predictive and provocative studies in human subjects when tested at up to 1% in pet. Cross-sensitization was observed between *p*-Phenylenediamine and *p*-aminophenol or *m*-phenylenediamine in a modified lymphocyte transformation test in guinea pigs.

Keratitis and corneal opacities were observed in rats that received up to 15% *p*-Phenylenediamine in formulation daily for up to 3 mo. No ocular irritation was observed in guinea pigs that received 2.5% *p*-Phenylenediamine. In rabbits, *p*-Phenylenediamine was moderately irritating when tested neat and weakly irritating at lower test concentrations.

Sensitization to p-Phenylenediamine has been observed around the globe, with sensitization rates varying greatly, independent of region or span of time. Using the US as an example, one retrospective study from 2001 to 2005 reported a positivity rate of 4.5%, while another reported a rate of 5.2% from 2006 to 2010, and a third reported a rate of 35.8% from 2001 to 2016. Greece has reported a positively rate as high as 52.5% (2010 to 2019) and India has reported a rate of 67.5% (dates not reported). In contrast, a German multicenter study reported a positivity rate of only 1.5% in 1994 to 1995. Additionally, numerous cases of adverse reactions to products containing *p*-Phenylenediamine have been reported in the published literature. *p*-Phenylenediamine intoxication through accidental or intentional oral ingestion of the dye has also been reported. Reactions to dark (black) henna tattoos/temporary tattoos containing *p*-Phenylenediamine have also been reported in large numbers, with most cases occurring outside of the US. Use of *p*-Phenylenediamine in dark (black) henna tattoos is not approved by the FDA. Skin depigmentation has been reported in numerous cases where patients were exposed to *p*-Phenylenediamine in hair dye products. Clinical reports of cross-sensitization reactions between *p*-Phenylenediamine and other hair dye ingredients, benzocaine, black rubber mix, and other chemicals have been documented.

The rate of sensitization of hairdressers has been studied. The OSHA PEL for 8-h work shifts and the NIOSH REL for 10-h TWA for exposure to *p*-Phenylenediamine are both 0.1 mg/m^3.

A conventional calculation by the SCCS for 2% *p*-Phenylenediamine under oxidative conditions determined the margin of safety to be 200. This calculation was based on the NOAEL of 8 mg/kg bw/d (from a 90-d oral rat study) and a SED of 0.04 mg/kg bw. In a toxicokinetic based approach performed by the SCCS, the margin of safety was calculated to be 23.3. This calculation used $AUC_{0-\infty}$ values from rat and human plasma concentration as systemic exposure doses. These values were based on data from a 6.45 mg/kg bw kinetic rat study and from 2% on-head application of *p*-Phenylenediamine in humans, respectively. The NOAEL of 8 mg/kg bw/d was also utilized.

The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer.

DISCUSSION

p-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate are reported to function as oxidative hair dyes in hair coloring products. Genotoxicity was observed in several in vitro studies, but was not observed in studies performed in vivo. Although mixed results were reported in the genotoxicity studies, the Panel noted the lack of toxicity in acute and repeated dose toxicity studies, the negative results in developmental and reproductive toxicity and carcinogenicity studies, and the short exposure time to these ingredients in hair dye formulations. Accordingly, the Panel determined that the data are sufficient to conclude that *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate are safe for use as hair dye ingredients in the present practices of use and concentration.

The Panel expressed particular concern over the practice of combining *p*-Phenylenediamine with henna (so-called dark or black henna) for use in temporary tattoos. The Panel also noted that use has been reported in eye makeup preparations. *p*-Phenylenediamine is a known dermal sensitizer in humans, and it is highly inappropriate for this ingredient to be used in products outside of hair dyes as evidenced by reports of severe adverse skin reactions to dark henna temporary tattoos. The Panel urged users to report adverse reactions to the FDA. The FD&C Act mandates that color additives must be approved by the FDA for their intended use before they are used. *p*-Phenylenediamine is not an approved color additive in cosmetics products, and thereby, such use is not permitted. Furthermore, use of *p*-Phenylenediamine outside of hair dying products is not within the purview of this Panel.

The Panel recognizes that hair dyes containing this ingredient, as coal tar hair dye products, are exempt from certain adulteration and color additive provisions of the FD&C Act when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures. The Panel considered concerns that such self-testing might induce sensitization, but agreed that there was not a sufficient basis for changing this advice to consumers at this time. The Panel noted that hair dyes, such as those containing *p*-Phenylenediamine, should not be applied to the eyebrows and eyelashes in that such use can result in lost or permanently damaged vision.

Articles reporting cases of depigmentation after exposure to *p*-Phenylenediamine have been identified in the published literature and the Panel noted that, clinically, this is a very uncommon or rare event and is not a significant safety concern. It was not clear whether the reactions observed in the reports were vitiligo induced by an allergic reaction, true chemical leukoderma, or are post-inflammatory events, and the nature of the reaction requires further study. Additionally, it was noted that the 48-h patch test for evaluating the skin irritation potential of hair dyes is sufficient for evaluating the skin depigmentation potential of *p*-Phenylenediamine.

In considering hair dye epidemiology data, the Panel concluded that the available epidemiology studies are insufficient to scientifically support a causal relationship between hair dye use and cancer or other toxicological endpoints, based on lack of strength of the associations and inconsistency of findings. Use of direct hair dyes, while not the focus in all investigations, appears to have little evidence of any association with adverse events as reported in epidemiology studies.

The Panel's respiratory exposure resource document (available at https://www.cir-safety.org/cir-findings) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be assessed by the Panel. Therefore, the Panel has found the data insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate are safe for use as hair dye ingredients in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Definitions, reported functions, and idealized structures of the ingredients in this safety assessment.[1, CIR Staff]

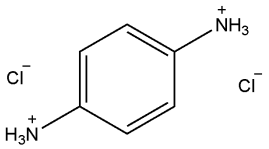
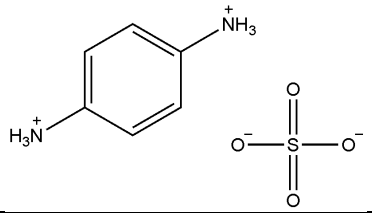
Ingredient & CAS No.	Definition	Function(s)
p-Phenylenediamine 106-50-3	*p*-Phenylenediamine is the aromatic amine that conforms to the structure in Figure 1.	hair colorant
p-Phenylenediamine HCl 624-18-0	*p*-Phenylenediamine HCl is the aromatic amine salt that conforms to the structure: 	hair colorant
p-Phenylenediamine Sulfate 16245-77-5 50994-40-6	*p*-Phenylenediamine Sulfate is the aromatic amine salt that conforms to the structure: 	hair colorant

Table 2. Chemical properties

Property	Value	Reference
	***p*-Phenylenediamine**	
Physical Form	white to light purple powder	7
Molecular Weight (g/mol)	108.14	7
Density (g/ml @ 22ºC)	0.726	5
Vapor pressure (mm Hg @ 20ºC)	7.5×10^{-5}	5
Melting Point (ºC)	139 - 141 142	7 5
Boiling Point (ºC)	267 274	7 5
Water Solubility (g/l @ 20ºC & pH 10)	31	5
Other Solubility (@ 22ºC)	ethanol: < 10% w/v DMSO: < 20% w/v	7 7
log P_{ow}	-0.31 (estimated) -0.84	7 5
Disassociation constant (pKa) (@ 20ºC)	6.22 (estimated)	5
UV Absorption (λ_{max}) (nm)	281.9	7
	***p*-Phenylenediamine HCl**	
Physical Form	White to gray or pink-beige powder	4
Molecular Weight (g/mol)	181.07	7
Melting Point (ºC)	140.7	4
Water Solubility (g/100 ml @ 22ºC for 24 h)	≥ 10; ≤ 20	4
Other Solubility (g/100 ml @ 22ºC for 24 h)	ethanol: < 10 DMSO: < 1	4
log P_{ow}	-0.3 (estimated) -0.84	4
	***p*-Phenylenediamine Sulfate**	
Physical Form	off-white powder	6
Density (g/ml @ 20ºC)	1.573	6
Vapor pressure (mmHg @ 25ºC)	3.1×10^{-6}	6
Water Solubility (g/l @ 30ºC)	3.71	6
log P_{ow} (@ 25ºC & pH 3)	0.856	6

Table 3. Frequency (2023/2006) and concentration (2022/2007) of use according to likely duration and exposure and by product category

	***p*-Phenylenediamine**				***p*-Phenylenediamine HCl**				***p*-Phenylenediamine Sulfate**			
	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2023[12]	**2006[4]**	**2022[13]**	**2007[4]**	**2023[12]**	**2006[4]**	**2022[13]**	**2007[4]**	**2023[12]**	**2006[4]**	**2022[13]**	**2007[4]**
Totals	**200**	**1497**	**0.98-3[≠]**	**2-4[†]**	**1**	**NR**	**NR**	**6[‡]**	**NR**	**NR**	**NR**	**6[‡]**
summarized by likely duration and exposure*												
Duration of Use												
Leave-On	*7*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Rinse-Off	*193*	*1497*	*0.98-3*	*NR*	*1*	*NR*	*NR*	*6*	*NR*	*NR*	*NR*	*6*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type**												
Eye Area	7	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	7	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Hair-Coloring	193	1497	0.98-3[≠]	2-4[†]	1	NR	NR	6[†]	NR	NR	NR	6[†]
Nail	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
as reported by product category												
Eye Makeup Preparations												
Eyeliner	1	NR	NR	NR								
Other Eye Makeup Preparations	6	NR	NR	NR								
Hair Coloring Preparations												
Hair Dyes and Colors (all types requiring caution statements and patch tests)	189	1478	0.98-3[≠]	2-4[†]	NR	NR	NR	6[‡]	NR	NR	NR	6[‡]
Hair Tints	1	16	NR	NR								
Hair Shampoos (coloring)	3	NR	NR	NR	1	NR	NR	NR				
Hair Lighteners with Color	NR	3	NR	NR								

NR – not reported

[≠] After dilution, maximum on-head use concentration 1%.

† 1-2% after dilution.

‡ 3% after dilution.

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.

**Likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

Table 4. Acute toxicity studies

Test Article	Vehicle	Animals/Group	Concentration/Dose	Protocol	LD_{50}/LC_{50}/Results	Reference
DERMAL						
p-Phenylenediamine in oxidative hair dye	not reported	Groups of 4 to 8 New Zealand rabbits, sex not reported	at least 5000 mg/kg bw	Dermal exposure to test material (10 ml), no further details provided	Mortality observed at 5000 mg/kg bw, no further details provided	[5]
p-Phenylenediamine applied as 40% aq. solution	none	New Zealand White rabbits, 1 female at low dose, 1 female and 1 male at high dose	5010 or 7940 mg/kg	Dermal exposure to test material for 24 h, no further details provided	LD_{50} > 7940 mg/kg, no mortalities reported; no further details provided	[5]
ORAL						
p-Phenylenediamine; purity not reported	water	Groups of 5 mice, strain and sex not reported	0, 35, or 70 mg/kg bw	Single oral dose; animals observed for clinical symptoms, with serial sacrifices for histopathology; no further details provided	A significant increase in serum CPK and aldolase was evident after 24 and 72 h; histopathology of animals sacrificed after 24 h showed rhabdomyolysis with areas of fresh necrosis; no further details provided	[5]
p-Phenylenediamine; 99.8% pure	sterile water	Female Sprague-Dawley Crl:OFA(SD) rats; number per group varied with dose	25, 50. 75, or 100 mg/kg	In accordance with OECD TG 420; 5 rats in 25 mg/kg dose group, 1 rat each in 50 and 100 mg/kg dose group, and 2 in 75 mg/kg dose group; rats received test material via gavage; observed up to 14 d after dosing; all animals underwent necropsy	Minimal lethal dose = 75 mg/kg; 1 rat in 100 mg/kg group died within 90 min of dosing and 1 rat in 75 mg/g died within 175 min; treatment-related clinical signs included marked subdued behavior and unsteady gait observed in the 50, 75, and 100 mg/kg dose groups; orange traces in the bedding, probably due to colored urine, observed (in which dose groups not stated) on day 0; no macroscopic findings observed in any animal	[5,7]
p-Phenylenediamine; purity not reported	water	14 dogs; details not provided	50, 80, or 100 mg/kg bw	Single oral dose; animals observed for clinical symptoms, with serial sacrifices for histopathology; no further details provided	Marked edema of the face, extremities, and external genitals, and painful muscle rigor observed; excessive increase in serum CPK observed in most animals, and animals in 80 mg/kg dose group had greatest increase; SGOT varied with the animals, and serum glutamic pyruvic transaminase (SGPT) did not increase significantly; histology of skeletal muscles showed massive necrosis, most pronounced in 80 mg/kg dose group; no further details provided	[5]
INHALATION						
p-Phenylenediamine; 99.5% pure	air	Groups of 10 male Crl:CD rats	0.07, 0.30, 0.54, 0.94, or 1.8 mg/l	Nose only inhalation study; rats exposed for 4 h; observed for 14 d for clinical symptoms	Calculated LC_{50} = 0.92 mg/l; deaths in at least half the animals observed at 0.94 and 1.8 mg/ml concentration groups, all deaths occurred within 48 h; at concentrations greater than 0.07 mg/l, rats had red nasal discharge; cyanosis observed at 1.8 mg/l; during observation period, rats at all concentrations had red ocular discharge or brown-stained fur; dose-dependent decrease in body weight for 3 d followed by weight gain	[5]

Table 5. Repeated dose toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
DERMAL							
1% solution of *p*-Phenylenediamine; purity not reported	25% ethanol	groups of 12 male guinea pigs, strain not reported	30 d	4 mg/kg	Dermal exposure with daily treatment for 30 d, open patch to clipped skin; skin enzymatic activities measured; concurrent vehicle control; no further details provided	No mortalities observed; activity of β-glucuronidase and acid phosphatase were significantly increased by test material over the control; activity of glutathione-transferase and glutathione peroxidase were significantly elevated; lipid peroxidation was increased significantly; significant increase observed in histamine	[5]
p-Phenylenediamine; purity not reported	double distilled water	groups of 5 male Sprague-Dawley rats	90 d	0, 1, 2, or 3 mg/kg bw/d	Dermal study with daily treatment for 90 d; test material applied to 1.5 cm^2 dorsal, clipped skin daily; open patch; body weights observed every 30 d until study end; hematological examination, enumeration of lymphocytes and abnormal/atypical cells in peripheral circulation, and assessment of spleen performed; no further details provided	LOAEL = 1 mg/kg bw/d; hemolytic anemia due to intravascular hemolysis and increased sequestration of damaged erythrocytes within splenic sinuses observed; sequestration events lead to increased deposition of the heme proteins which cause histopathological changes to the spleen; no other endpoints were described	[5]
p-Phenylenediamine in a hair dye formulation	none	30 male guinea pigs, strain not reported	4 mo	not reported	Test material applied weekly according to dye instructions, alternating right and left flank; feed consumption and clinical signs recorded weekly; blood examinations made every 4 wk; animals killed after 4 mo; microscopic examination of heart, large blood vessels, lung spleen, liver, and adrenal; no further details provided	Mild erythema observed in 3 guinea pigs; no other pathological, blood, or microscopic changes observed	[5]
ORAL							
p-Phenylenediamine; purity not reported	deionized water	groups of 10 male and 10 female Crl: CD (SD) BR (VAF plus) rats	14 d	0, 5, 10, 20, or 40 mg/kg/d	Range-finding study in accordance with OECD TG 408; rats received test material (10 ml/kg bw) daily via gavage; no further details provided	NOAEL < 5 mg/kg bw/d; no treatment-related effects noted on mortality, clinical signs of toxicity, body weight gains, feed consumption, hematological parameters, or macroscopic observations at necropsy; increased lactate dehydrogenase and CPK levels observed in both sexes at 5 mg/kg or greater; increased mean absolute and relative liver weights in 40 mg/kg males; increased mean relative thyroid weights in 10 mg/kg or greater females; minimal myodegeneration noted in skeletal muscle of three 40 mg/kg females	[9]
p-Phenylenediamine; purity not reported	dietary feed	groups of 5 male and 5 female Fischer 344 rats	7 wk	0, 681, 1000, 1470, 2150, or 3160 ppm	Short-term oral toxicity study; no further details provided	NOEL for females = 681 ppm, NOEL for males = 1000 ppm; decreased weight gain for females was $\geq$ 1000 ppm and for males was $\geq$ 2150 ppm; no other effects were described	[5]
p-Phenylenediamine; purity not reported	dietary feed	groups of 10 - 11 F344 rats per sex	12 wk	0, 0.05, 0.1, 0.2, or 0.4%	Subchronic oral toxicity study; rats killed after 12 wk; main organs weighed and examined macroscopically and histologically	Dose-dependent growth retardation observed in both sexes, especially in the 0.4% group; liver-to-body weight and kidney-to-body weight ratios in 0.4% group higher when compared to control; 9 males and 1 female in the 0.4% group died before study end	[26]

Table 5. **Repeated dose toxicity studies**

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
p-Phenylenediamine; purity not reported	deionized water	groups of 15 male and 15 female Crl: CD (SD) BR rats	90 d	0, 2, 4, 8, or 16 mg/kg/d	Subchronic oral toxicity study in accordance with OECD TG 408; rats received test material (10 ml/kg bw) daily via gavage; rats examined daily for mortality and clinical signs of toxicity; feed consumption and body weight recorded weekly; ophthalmoscopic examination was performed at pre-study and at week 13 in control and high-dose rats; urine and blood samples were collected at weeks 4 and 13 from all rats; all rats killed after 13 wk and necropsied; macroscopic and microscopic examinations performed	NOEL = 4 mg/kg/d, NOAEL = 16 mg/kg/d; no treatment-related mortalities or clinical signs of toxicity observed; no adverse effects on feed consumption, body weights, or body weight gain observed; no treatment-related ophthalmologic, hematology, blood chemistry, or urinalysis changes observed; mean absolute and body-weight-related liver weights significantly increased for 8 and 16 mg/kg/d males; absolute and body weight-related kidney weights were increased for 8 and 16 mg/kg/d females; no associated histopathological changes noted; no treatment-related macroscopic or microscopic findings recorded; minimal myodegeneration on skeletal muscle in 1 male and 1 female of the 16 mg/kg/d group	[5,9]
p-Phenylenediamine; purity not reported	Sterile water	Groups of 10 male and 10 female ccl:CD BR rats	13 wk	0, 4, 8, or 16 mg/kg bw/d	Oral neurotoxicity study; performed in similar manner as above with the addition of neurotoxicity evaluations performed before and after 4, 8, and 13 wk of dosing according to a test battery that included motor activity and functional battery assessments	NOEL = 8 mg/kg bw/d, NOAEL = 16 mg/kg bw/d; no treatment-related mortalities or clinical signs of toxicity observed; feed consumption and body weight gains in treated groups comparable with controls; no ocular effects observed; at 16 mg/kg bw/d, increased incidence of wet chin in both sexes and wet inguen and/or wet perineum was observed in females; neuropathology evaluations did not reveal abnormalities within the nervous system of skeletal muscle; effects observed at 16 mg/kg bw/d considered to be pharmacological responses	[9]

Table 6. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
DERMAL						
p-Phenylenediamine; purity not reported	not reported	groups of 40 Sprague-Dawley rats of each sex	2, 3, or 4% in formulation and mixed with 6% hydrogen peroxide	Multigeneration reproduction study; test materials (0.5 ml) applied topically twice weekly throughout growth, mating, gestation and lactation phases of F_0 parents to the weaning of the F_{1a} and F_{2b} litters; rats in the F_0 generation received test material until 100 d old; test site was 1 in. in diameter; open patch; no further details provided	No adverse effects on reproduction; no adverse effects on fertility of males or females, on gestation, lactation, or weaning indices; average number weaned per litter and mean body weights of weanlings comparable among treated and control groups	[27]
p-Phenylenediamine; purity not reported	double distilled water	groups of 10 Sprague-Dawley male rats	0, 1, 2, or 3 mg/kg/d	Rats (~130 g) painted on clipped dorsal side with test material for 90 d; open patches; body weights recorded at pre-dosing and prior to necropsy; weight of both testes and epididymis recorded at necropsy; histological examination performed on testes; no further details provided	Significant body weight decrease ($p < 0.05$) in 3 mg/kg group; in 2 and 3 mg/kg dose group, a significant decrease ($p < 0.05$, 0.01, respectively) in absolute testes weight, but not in relative testes weight; no differences observed in epididymal weight between control and treated group; also in the 2 and 3 mg/kg dose group, significant decrease in total sperm count ($p < 0.05$ for both) and a significant increase ($p < 0.05$, 0.01, respectively) in the percentage of abnormal sperm morphology also observed; elevation of lipid peroxidation product in the testicular tissue ($p < 0.01$) indicated potential oxidative stress; morphological abnormality in testicular tissue observed in groups treated with 2 and 3 mg/kg	[28]
ORAL						
p-Phenylenediamine; purity not reported	DMSO with water	groups of 5 female ICR mice	0, 25, or 50 mg/kg	Reproductive toxicity study; mice received test material via gavage for 10 d; ovaries and oocytes were analyzed after exposure period was complete; additional control and treated female mice (number not reported) were mated with untreated males after the dosing period and fertilized eggs were analyzed	Test material affected meiotic capacity of oocytes and fertilization potential; damage to the spindle/chromosome structure was observed; development and maturation of the oocytes was impaired; the test material also compromised the dynamics of cortical granules and ovastacin; sperm receptors on the egg membrane were also impaired in treated oocytes, leading to fertilization failure; treated oocytes exhibited abnormal mitochondrial function, which resulted to degeneration, apoptosis, and increased ROS levels	[29]

Table 6. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
p-Phenylenediamine; 99.8% pure	sterile water	groups of 25 mated female Sprague-Dawley rats	0, 5, 10 or 20 mg/kg/d	Developmental toxicity study in accordance with OECD TG 414; rats received the test material via gavage once daily on gestation days 6-19; clinical condition, body weights, and feed consumption monitored in dams during dosing period; dams underwent caesarean examination on gestation day 20 and litter parameters were recorded	Maternal NOEL = 5 mg/kg/d; developmental NOAEL = 10 mg/kg/d; test material was considered non-embryo-fetotoxic; in dams; no unscheduled deaths or clinical signs of toxicity; slightly transient lower mean gestation body weight gain noted in the 10 and 20 mg/kg/d dose groups during first 3 d of treatment; no effect on maternal feed consumption in any dose group and no treatment-related macroscopic findings at necropsy; no differences in pre- or post-implantation data between treated and control groups except for equivocal increase incidence of early resorptions in the high dose group; mean live litter sizes comparable between treatment and control groups; mean fetal weight and mean gravid uterus weight slightly lower in high dose dams than in other groups (not statistically significant); fetal sex ratio comparable between groups; no malformed fetuses observed; incidences of fetuses with morphological anomalies or variations did not suggest any treatment-related adverse effects	[5]

Table 7. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
			IN VITRO			
p-Phenylenediamine; purity not reported	up to 1 µmol/plate	not reported	*Salmonella typhimurium* strain TA98	Ames test, with and without metabolic activation	Mutagenic with metabolic activation	[5]
p-Phenylenediamine; purity not reported	1 or 5 µmol/plate	not reported	*S. typhimurium* strain TA98	Ames test, with metabolic activation only	Mutagenic with metabolic activation	[5]
p-Phenylenediamine; purity not reported	up to 0.46 mM	not reported	*S. typhimurium* strain TA98	Ames test, with and without metabolic activation	Mutagenic with metabolic activation, not mutagenic without metabolic activation	[5]
p-Phenylenediamine; purity not reported	0.8 - 80 mM	not reported	*S. typhimurium* strain TA98, TA100, YG1024, TG1029	Ames test, with and without metabolic activation	Not mutagenic; cytotoxic above 52.8 mM	[5]
p-Phenylenediamine; purity not reported	up to 1500 mM	not reported	*S. typhimurium* strain TA98 and YG1024	Ames test, with and without metabolic activation	Not mutagenic	[5]
p-Phenylenediamine; purity not reported	25-250 µg/plate	not reported	*S. typhimurium* strain TA98 and TA1538	Ames test, with metabolic activation	Mutagenic with metabolic activation	[5]
p-Phenylenediamine; 97% pure	67 - 1076 µg/plate	DMSO	*S. typhimurium* strains TA98 and TA100	Ames test, with and without metabolic activation	Mutagenic to strain TA98 with metabolic activation	[33]
p-Phenylenediamine; purity not reported	up to 1000 µg/plate	not reported	*S. typhimurium* strains TA98, TA100, TA1535, TA1537	Ames test, with and without metabolic activation	Not mutagenic	[5]
p-Phenylenediamine; purity not reported	up to 1000 µg/ml of agar	not reported	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538, G46, C3076, D3052 and *Escherichia coli* strains WP2 and WP2uvrA-	Ames test, with and without metabolic activation	Mutagenic for strains TA98 and TA1538 with metabolic activation, not mutagenic for the remaining strains with or without metabolic activation	[5]
p-Phenylenediamine; purity not reported	up to 2000 µg/plate	not reported	*S. typhimurium* strain TA98	Ames test, with and without metabolic activation	Mutagenic with metabolic activation, not mutagenic without metabolic activation; cytotoxic at 2000 µg/plate	[5]
p-Phenylenediamine; purity not reported	up to 3000 µg/plate	DMSO	*S. typhimurium* strains TA98 and TA100	Ames test, with and without metabolic activation	Mutagenic in strain TA98 with metabolic activation; not mutagenic in strain TA100	[31]
p-Phenylenediamine; purity not reported	up to 3000 µg/plate	DMSO	*S. typhimurium* strains TA98, TA98NR, TA100, TA100NR	Ames test, with and without metabolic activation	Weakly mutagenic to strain TA98NR with metabolic activation and strain TA100NR without metabolic activation when compared to the control	[30]
p-Phenylenediamine; purity not reported	up to 5000 µg/plate	not reported	*S. typhimurium* strains TA102 and TA2638 and *E. coli* strains WP2/pKM101 and WP2uvrA/pM101	Ames test, with and without metabolic activation	Mutagenic without metabolic activation in TA102 and in both *E. coli* strains; not mutagenic with metabolic activation in all strains tested	[5]
p-Phenylenediamine; purity not reported	up to 10,000 µg/plate	DMSO	*S. typhimurium* strains TA98 and TA100	Ames test and preincubation protocols, with and without metabolic activation	Not mutagenic	[32]
p-Phenylenediamine; purity not reported	50,000-100,000 µg/plate	not reported	*S. typhimurium* strain TA1538	Ames test, with and without metabolic activation	Mutagenic with metabolic activation; not mutagenic without metabolic activation	[5]
p-Phenylenediamine; purity not reported	up to 5 mg/ml	DMSO	*S. typhimurium* strain TA1535/pSK1002	Umu post-treatment assay, with and without metabolic activation	Mutagenic with metabolic activation	[39]
p-Phenylenediamine; purity not reported	up to 190.4 µg/ml	distilled water	*E. coli* strains B, Bb, CR63, and K12 (λh)	Bacteriophage T4D assay	Not mutagenic	[40]

Table 7. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
p-Phenylenediamine; purity not reported	not reported	acetone	*E. coli* strain B/r.WP2(lambda)_	Microscreen assay, with and without metabolic activation	Mutagenic without metabolic activation; the induction of lambda prophage results from depression of the bacterial SOS system, which becomes activated when DNA is damaged; no further details provided	5
p-Phenylenediamine; purity not reported	up to 0.1%	not reported	*S. cerevisiae* strain D3	Mitotic recombination assay, with and without metabolic activation	Not mutagenic; cytotoxic at 0.1%	5
p-Phenylenediamine; purity not reported	up to 0.3 mM	not reported	*S. cerevisiae*, strain not reported	Gene mutation assay, with and without metabolic activation	Mutagenic with and without metabolic activation; test material exhibited dose-dependent mutagenic activity	5
p-Phenylenediamine; purity not reported	up to 87 µg/ml	DMSO	CHO cells	Chromosome aberrations assay, without metabolic activation	Dose-related increase in chromosomal aberrations observed, with 27% aberrant cells noted at the highest concentration tested	31
p-Phenylenediamine; purity not reported	up to 87 µg/ml	DMSO	CHO cells	Chromosome aberrations assay, without metabolic activation	Dose-related increase in chromosomal aberrations, with 28% aberrant cells noted at the highest concentration tested	30
p-Phenylenediamine; purity not reported	0.4 mM	not reported	CHO cells	Sister chromatid exchange assay in accordance with OECD TG 479, with and without metabolic activation	Genotoxic, no further details provided	5
p-Phenylenediamine; purity not reported	3.1 - 50 µg/ml	not reported	CHL cells	Micronucleus test, with and without metabolic activation	Genotoxic without metabolic activation; at 50 µg/ml, more than 10% of cells showed micronuclei	5
p-Phenylenediamine; purity not reported	0.0005 - 1 µmol/ml	DMSO	rat hepatocytes	Unscheduled DNA synthesis assay, without metabolic activation	Not genotoxic without metabolic activation	5
p-Phenylenediamine; "highest purity available"	0, 2, 5, 10, 20, or 40 µg/ml	not reported	SV-40 immortalized human uroepithelial cell line	Single cell gel/comet assay; no further details provided	Increased expression of mutant p53 and COX-2 proteins; dose-dependent reduction in cell viability; no further details provided	5
p-Phenylenediamine HCl; purity not reported	up to 1000 µg/plate without hydrogen peroxide; up to 25 µg/plate with hydrogen peroxide	sterile water when tested without hydrogen peroxide; 2% ammonia hydroxide when tested with hydrogen peroxide	*S. typhimurium* strain TA98	Ames test, with and without metabolic activation	Weakly mutagenic with metabolic activation without hydrogen peroxide; mutagenic with metabolic activation with hydrogen peroxide; not mutagenic without metabolic activation with or without oxidation	34
p-Phenylenediamine HCl; 99.3% pure	up to 5000 µg/plate	purified water	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102	Ames test, with and without metabolic activation; pre-incubation study with only strain TA98, with metabolic activation	Statistically significant ($p < 0.01$) increase in number of revertants in strain TA100 at 1000 µg/plate without metabolic activation, however, no dose response relationship; statistically significant increase in number of revertants in strain TA98 at 1000 µg/plate ($p < 0.01$) and at 5000 µg/plate ($p < 0.005$); in pre-incubation, statistically significant, dose-related increase in number of revertants	35
p-Phenylenediamine HCl; 96.1% pure	up to 6666 µg/plate	distilled water	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and *E. coli* strain WP2 urA	Ames test, with and without metabolic activation	Mutagenic in at least one *S. typhimurium* strain with metabolic activation; not possible to determine whether test substance was mutagenic in *E.coli* strain	38

Table 7. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
p-Phenylenediamine HCl before and after treatment with hydrogen peroxide; oxidized mixture of *p*-Phenylenediamine HCl with *m*-phenylenediamine HCl or *o*-phenylenediamine HCl	up to 10 µg/plate	DMSO	*S. typhimurium* strain TA98	Ames suspension assay, with and without metabolic activation; test performed on test materials before and after treatment with hydrogen peroxide	Mutagenicity of *p*-Phenylenediamine HCl alone slightly enhanced by hydrogen peroxide in the presence of metabolic activation; hydrogen peroxide-oxidized mixtures classified as potent mutagens with metabolic activation	[36]
p-Phenylenediamine HCl (55 mM), resorcinol (66 mM), and hydrogen peroxide (3%)	up to 5.5 µmol/plate	DMSO	*S. typhimurium* strain TA97, TA98, TA100	Ames test, with and without metabolic activation	Oxidative mixture not mutagenic; however, same oxidative mixture without resorcinol was mutagenic	[37]
p-Phenylenediamine HCl; purity not reported	Up to 6.5 µg/ml without metabolic activation; up to 250 µg/ml with metabolic activation	distilled water	L5178 mouse lymphoma cells	Forward mutation assay, with and without metabolic activation	Dose-related increase in mutagenicity in 2 out of 3 trials, with and without metabolic activation	[41]
p-Phenylenediamine HCl; purity not reported	up to 10 µg/ml without metabolic activation; up to 400 µg/ml with metabolic activation	distilled water	L5178Y mouse lymphoma cells	Forward mutation assay, with and without metabolic activation	Significant increases in mutant frequency, with and without metabolic activation; responses were usually larger without metabolic activation and occurred at less than 1/10 the concentrations required with metabolic activation	[42]
p-Phenylenediamine HCl; 99.3% pure	up to 80 µg/ml without metabolic activation; up to 1000 µg/ml with metabolic activation	purified water	L5178Y mouse lymphoma cells	Gene mutation assay at the *hprt* locus in accordance with OECD TG 476	Not mutagenic	[35]
p-Phenylenediamine HCl; 99.3% pure	test 1: 3.73, 30, 80 µg/ml without metabolic activation and 500, 900, 1600 µg/ml with metabolic activation test 2: 50, 100, 125 µg/ml without metabolic activation and 400, 1400, 2000 µg/ml with metabolic activation	purified water	human lymphocytes	Micronucleus test in accordance with OECD TG 487; test 1 had 24 h stimulation, 20 h treatment and 28 h recovery without metabolic activation and 24 h stimulation, 3 h treatment and 35 h recovery with metabolic activation; test 2 had 48 h stimulation, 20 h treatment and 28 h recovery without metabolic activation and 48 h stimulation, 3 h treatment and 45 h recovery with metabolic activation	Genotoxic; test material induced micronuclei in test 1 with metabolic activation and in test 2 with and without metabolic activation	[35]
IN VIVO						
p-Phenylenediamine; purity not reported	0, 10.8, 21.6, or 32.4 mg/kg	not reported	groups of 4 male and female mice, no further details provided	Micronucleus test; mice received test material intraperitoneally; no further details provided	Not genotoxic; test material did not induce chromosomal abnormalities; no further details provided	[5]
p-Phenylenediamine; purity not reported	10 mg/kg	not reported	rat, no further details provided	Micronucleus test; rats received 2 oral doses of 10 mg/kg test material; no further details provided	Not genotoxic; no further details provided	[5]
p-Phenylenediamine; 99.8% pure	25, 50, or 100 mg/kg bw	deionized water	groups of Wistar rats, 5 rats per sex	Micronucleus test in rat bone marrow cells in accordance with OECD TG 474; rats received a single oral dose of test material and were killed 24 h later	Not genotoxic; increases in micronucleated bone marrow cells within range of historical controls and were not considered biologically relevant	[7]

Table 7. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
p-Phenylenediamine; purity not reported	0 or 300 mg/kg bw	not reported	groups of 5 male and 5 female Sprague-Dawley rats	Micronucleus test in accordance with OECD TG 474; rats received 2 equal doses of 300 mg/kg bw test material 24 h apart via gavage; rats killed 6 h after last dose; no further details provided	Not genotoxic; no further details provided	[5]
p-Phenylenediamine; 99.8% pure	50 or 100 mg/kg bw	deionized water	groups of 3 male Wistar Hanlbm: WIST (SPF) rats	Unscheduled DNA synthesis in rat hepatocytes in accordance with OECD TG 486; rats received single oral dose of test material and were killed 2 or 16 h after dosing	Not genotoxic; no increased mean net nuclear grain count observed in the hepatocytes when compared to the negative controls	[7]
p-Phenylenediamine; purity not reported	0, 25, 50, or 100 mg/kg bw/d	0.9% physiological saline	groups of 3-5 male Sprague-Dawley Crl:CD (SD) IGS rats	Comet assay in accordance with OECD TG 489; rats received test material via oral gavage; rats dosed 3 times 24 and 21 h apart; 3 h after final treatment; rats were killed and liver and stomach were sampled	Not genotoxic; no increases in DNA damage observed in liver and stomach	[5]
p-Phenylenediamine HCl; purity not reported	0, 25, 50, or 100 mg/kg	distilled water	groups of 5 male CD-1 mice	Micronucleus test; mice received single intraperitoneal dose of test material; mice then killed and bone marrow smears prepared; polychromatic erythrocytes scored for incidence of micronuclei	Not genotoxic; test material did not induce micronuclei	[43]

Table 8. Carcinogenicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
DERMAL							
p-Phenylenediamine; purity not reported	acetone	30 female Sutter mice	20 wk	5%	One drop of test material was applied to the backs of mice twice weekly; at study end, the surviving mice were studied for papillomas or carcinomas; concurrent vehicle control group used; no further details provided	No papillomas or carcinomas reported; mortality observed in 19 of 30 mice by 20 wk; no further details provided	[5]
p-Phenylenediamine; purity not reported	not reported	4-5 rats per group; no further details provided	up to 8 mo	12.5 or 20 mg/kg	Rats received 12.5 mg/kg test material subdermally daily for 8 mo or 20 mg/kg for 4 mo; no further details provided	In the 12.5 mg/kg dose group, tumors were observed 2 of 5 rats in the month 7; no tumors observed in the 20 mg/kg dose group; no further details provided	[5]
p-Phenylenediamine HCl; purity not reported	2% ammonium hydroxide	10 male and 10 female Wistar rats per group	18 mo	5%	Test material and 6% hydrogen peroxide (1:1 ratio) applied topically (0.5 ml) to shaved back skin once per wk; another group received the test materials by subcutaneous injection (0.1 ml) in the hip; control group received vehicle only	In female rats, a statistically significant incidence (> 50%, $p < 0.05$) of mammary gland tumors was observed in treated rats, mammary gland tumors not observed in males; 1 female also had a soft tissue tumor (fibromatosis); 40% of male rats observed with tumors of the liver, kidney, adrenal gland, thyroid gland, urinary bladder, and lung, results in males were not statistically significant	[34]
ORAL							
p-Phenylenediamine; purity not reported	dietary feed	male Sprague-Dawley rats; number not reported	9 mo	not reported	Rats received test material daily in feed for 9 mo; no further details provided	Not carcinogenic; no further details provided	[5]
p-Phenylenediamine; purity not reported	dietary feed	Groups of 63 to 66 F344 rats of each sex	80 wk	0.05 or 0.1%	Animals fed test material in diet ad libitum for 80 wk; control group of 24-25 rats of each sex received regular diet; body weights and feed consumption recorded weekly; animals surviving until study end underwent hematological analysis; macroscopic and histological examinations performed	Not carcinogenic; body weights of 0.1% female rats less than controls, but no differences noted at 0.05% in females or in either male dose group; highest incidence of neoplastic lesions in both sexes was that of pheochromocytomas of the adrenal gland with incidence of 27.8% in the 0.1% males, 22.9% in the 0.05% males, and 31.6% control males (females had lower incidences in all groups than males); other neoplastic lesions included hyperplasia of the forestomach in males, a fibroadenoma of the mammary gland in a female, a fibroma of the skin in a male, lymphomas in females, and ductal hyperplasia of the pancreas in a female; incidences of these lesions were not significantly different in among the groups; other effects observed, which included hemorrhage of the pituitary gland, fatty degeneration of the liver, fibrosis of the pancreas, and pneumonia, were not significantly different in different groups; no marked changes of the thyroid gland observed in any rats	[26]

Table 8. Carcinogenicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
p-Phenylenediamine HCl; purity not reported	not reported	groups of 22 female pregnant NMRI mice	daily treatment on days 10-19 of gestation; observed for 137 wk	0 and 30 mg/kg	Mice treated via gavage during days 10-19 of gestation; select mice killed at 27 and 51 wk, total observation time 137 wk; F_1 generation observed for carcinogenicity; no further details provided	Not carcinogenic; no adverse effects o body weights or survival observed in parental or F_1 animals; tumor incidence in treated animals comparable to controls	[5,7]
PARENTERAL							
p-Phenylenediamine; > 99% pure	soybean oil	51 male and 55 female NMRI mice per dose group	daily treatment on days 5-9 of life followed by 130 wk observation	30 mg/kg	Mice received an intraperitoneal injection of test material once daily on days 5-9 of life; 10 mice from treated group were killed 26 and 52 wk after treatment began; remainder of mice died or were killed in moribund state; necropsy and histological examination performed; vehicle controls (49 male and 43 female) received 10 mg/kg/d soybean oil and positive controls (42 males and 27 females) received 300 mg/kg/d urethane	Not carcinogenic; no treatment-related effects on body weight or survival observed; tumors observed in 30.1% of treated animals, with most common tumor types being benign lymphoma and alveolar adenoma; tumor incidence in vehicle control mice was 18.2%, positive control was 82.1%	[5,9]
p-Phenylenediamine HCl; purity not reported	tricaprylin	Lab A: 10 or 20 Strain A mice per sex per group and 54 male and 54 female controls Lab B: 30 male Strain A mice	8 wk	Lab A: 12.5 or 25 mg/kg Lab B; 6.4, 16, or 32 mg/kg	Mice received intraperitoneal injections 3 times/wk	Not carcinogenic; in Lab A and Lab B, the percentage of mice with tumors and the number of survivors were not significantly different from the vehicle control groups; results for Lab A female mice were equivocal, however, number of tumors per mouse was significantly different ($p \leq 0.05$) only at 25 mg/kg when compared to vehicle control	[45]
p-Phenylenediamine HCl; purity not reported	2% ammonium hydroxide and 1.8% sodium chloride	10 male and 10 female Wistar rats per group	18 mo	5%	Test material and 6% hydrogen peroxide (1:1 mixture) injected subcutaneously (0.1 ml) every other wk; control group received vehicle only	In female rats, a statistically significant incidence (> 50%, $p < 0.05$) of mammary gland tumors was observed in treated rats, mammary gland tumors not observed in males; female rats also had significant incidence of uterine tumors and malignant and benign soft tissue tumors (43% and 57%, respectively, $p < 0.05$), two of the of soft tissue tumors were at injection site; one male rat observed with both malignant tumors of the lung and thyroid gland	[34]
TUMOR PROMOTION							
p-Phenylenediamine; 99.5% pure	dietary feed	groups of 25 male F344/DuCrj rats	6 wk	110, 330, or 1000 ppm	Study of the modifying effects of the test material on liver carcinogenesis; rats received test material 2 wk after administration of single intraperitoneal dose of *N*-nitrosodiethylamine; positive control was 3'-methyl-4-dimethylaminobenzene (600 ppm) in feed; partial hepatectomy in all rats occurred after 1 wk of dosing	Test material did not significantly increase γ-glutamyl transpeptidase positive foci that were observed 3 wk after *N*-nitrosodiethyl-amine initiation; slight decrease in body weight observed in all rats that received test material at all dose levels; significant increases in relative liver weight reported in 1000 ppm dose group; positive control yielded expected results	[47]

Table 8. Carcinogenicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
p-Phenylenediamine HCl; purity not reported	saline	14 male Fischer 344 rats received test material, additional 18 rats received vehicle alone	5 wk	40 mg/kg single dose	Medium-term bioassay system assessed tumor promotion based on induction of glutathione 5-transferase (placental form) positive liver cell foci in rats; single dose intragastrically 12 h after partial hepatectomy; animals fed a basal diet for 2 wk, after which they were placed on a diet containing 0.015% 2-acetylaminofluorene for 2 wk; 3 wk after partial hepatectomy, all animals received carbon tetrachloride; after week 5, survivors were killed and livers were excised and prepared for the immunohistochemical examination of glutathione 5-transferase positive foci.	*p*-Phenylenediamine HCl did not cause a significant increase in the number of glutathione 5-transferase positive foci when compared to controls	[46]

Table 9. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION						
IN VITRO						
p-Phenylenediamine Sulfate; purity not reported	no vehicle	tested neat	human reconstructed epidermis	EpiSkin™ reconstructed human epidermis model in accordance with OECD TG 439	Predicted to be not irritating	[6]
ANIMAL						
p-Phenylenediamine; purity not reported	25% ethanol	0.9%	guinea pigs, details not provided	dermally administered at 0.1 ml; no further details provided	Pathomorphological lesions observed, but later disappeared; significant lipid peroxidation activity of skin homogenate observed, but superoxide dismutase not affected by treatment; histamine content increased initially, but reduced during recovery; no further details provided	[5]
p-Phenylenediamine; purity not reported	distilled water	0.5 and 1%	5 and 10 male albino guinea pigs	Primary irritation test; 0.5% solution applied to abraded, shaved skin of 5 animals, 1% solution applied to intact, shaved skin of 10 animals	Slightly irritating to abraded skin at 0.5%; moderately irritating to intact skin at 1%	[5]
p-Phenylenediamine; 100% pure	13% guinea pig fat in 50/50 acetone/dimethoxy ethane	5 and 10%	10 male albino guinea pigs	Primary irritation test; applied to shaved, intact shoulder skin; observations were made at 24 and 48 h after treatment	Not irritating	[5]
p-Phenylenediamine; purity not reported	dimethyl phthalate	2.5 and 25%	10 male Dunkin-Hartley guinea pigs	Primary irritation test; applied to shaved, intact shoulder skin; observations were made at 24 and 48 h after treatment	Mild skin irritant; no irritation observed at 2.5%; mild to no irritation observed at 25%; range-finding test determined test material was a moderate skin irritant at 70% (no further details provided)	[5]
p-Phenylenediamine; purity not reported	13% guinea pig fat in 50/50 acetone/dimethoxy ethane	10 and 25%	10 male albino guinea pigs	Primary irritation test; applied to shaved, intact shoulder skin; observations were made at 24 and 48 h after treatment	Not irritating	[5]

Table 9. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
p-Phenylenediamine; purity not reported	acetone, 50% aq. solution of Carbowax 1500, or petrolatum	1% in acetone 1 and 15% in Carbowax, 2% and 30% in pet	guinea pigs, details not provided	details not provided	Dermatitis observed at 2% in petrolatum that may be allergy response; at 30% in petrolatum, inflammatory response was difficult to determine as allergy or primary irritation; allergic reaction observed at 1% and 15% in Carbowax 1500; similar effect noted at 1% in acetone; no further details provided	[5]
p-Phenylenediamine; purity not reported	distilled water	3 and 30%	10 male albino guinea pigs; range-finding study conducted with 3 male albino guinea pigs	Primary irritation test; 0.05 ml applied to shaved, intact shoulder skin; observations made at 24 and 48 h after treatment	Not irritating	[5]
p-Phenylenediamine; purity not reported	acetone:dimethyl phthalate (1:9)	3 and 30%	10 male Dunkin-Hartley guinea pigs	0.5 ml applied to shaved shoulder skin and lightly rubbed in; observations were made at 24 and 48 h after treatment	Not irritating; no edema or erythema observed	[5]
p-Phenylenediamine; purity not reported	not reported	2.5% solution containing 0.05% sodium sulfite	rabbits; details not provided	Draize irritation study; test material applied to abraded or intact rabbit skin under gauze patch; no further details provided	Mildly irritating; primary irritation index = 0.3 out of a maximum of 8; no further details provided	[7]
p-Phenylenediamine; purity not reported	not reported	5% and in 4 formulations at unknown concentrations	rabbits; details not provided	details not provided	Test material at 5% considered a weak irritant; no irritation observed in the 4 formulations containing *p*-Phenylenediamine; no further details provided	[5]
p-Phenylenediamine; purity not reported	petrolatum, oil (type not specified), or water	2.5 and 25% in petrolatum, 10% in oil, and 50% in water	rabbits; details not provided	irritation study; no further details provided	Irritation indices ranged from 1.4 to 3.4; 2.5% was slightly irritating while 10% to 50% was moderately irritating; irritation reversible; no further details provided	[5]
p-Phenylenediamine; 99.97% pure	no vehicle	neat	6 albino rabbits; sex not reported	irritation study; test material applied to shaved back of rabbits and occluded; observations made at 4, 24, and 48 h after application	Not corrosive	[5]
SENSITIZATION						
ANIMAL						
p-Phenylenediamine; 97% pure	acetone:olive oil (4:1)	0, 0.05, 0.1, 0.25, 0.5, and 1%	groups of 4 female CBA/Ca mice	LLNA; 4 independent analyses performed in parallel in each of 2 independent laboratories	Sensitizing responses occurred at concentration of 0.25% or greater; EC_3 values ranged from 0.06 - 0.20%	[52]
p-Phenylenediamine; 100% pure	acetone:olive oil (4:1)	0, 0.05, 0.25, and 1.25% (w/v)	groups of 5 female CB/J mice	LLNA	Sensitizing; SI were 2.6, 10.4, and 16.1 for 0.05, 0.25, and 1.25%, respectively; EC_3 value was 0.06%; controls yielded expected results	[5,7]
p-Phenylenediamine; purity not reported	acetone:olive oil or dimethylformamide	0, 0.5, 1, and 2%	groups of 3 CBA/Ca mice; sex not reported	LLNA	Sensitizing; SI were 3.45, 5.27, and 4.77 for 0.5, 1, and 2%, respectively; no further details provided	[5]

Table 9. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
p-Phenylenediamine; purity not reported	dimethylacetamide:acetone: ethanol (4:4:3)	0, 0.5, 1, 2, and 5%	groups of 3 Dunkin-Hartley-Pirbright guinea pigs; sex not reported	LLNA; cultures maintained for 24 and 48 h, 48 h cultures in the presence or absence of human recombinant IL-2	Sensitizing; SI after 48 h were 1.03, 0.91, 1.32 and 2.04 for 0.5, 1, 2, and 5%, respectively; SI after 48 h and with human recombinant IL-2 were 0.94, 0.97, 7.40, and 9.75 for 0.5, 1, 2, and 5%, respectively; no further details provided; study not considered reliable by ECHA as guinea pigs were used in place of mice, no positive controls were used, little data on animals were provided, and no criteria presented for positive result	[5]
p-Phenylenediamine; purity not reported	acetone:olive oil (4:1)	0, 2.5, 5, and 10%	groups of 4 CBA/Ca mice; sex not reported	LLNA; 4 independent laboratories performed analyses in parallel	Sensitizing; SI for 2.5% ranged from 6.5-21.0; SI for 5.0% ranged from 16.5 to 26.0; SI for 10% ranged from 23.3 to 75.3; no further details provided	[5]
p-Phenylenediamine; purity not reported	not reported	0, 2.5, 5, and 10%	groups of 4 mice; no further details provided	LLNA	Sensitizing; no further details provided	[5]
p-Phenylenediamine; purity not reported	not reported	0, 2.5, 5, and 10%	groups of 3 mice; no further details provided	LLNA	Sensitizing; no further details provided	[5]
p-Phenylenediamine; 99% pure	induction: physiological saline challenge: physiological saline (intradermal) and pet. (epidermal)	induction: 0.1% challenge: 1%	Pirbright white, Dunkin-Hartley and Himalayan spotted guinea pigs, 5 males and 5 females per strain	Guinea pig optimization test; guinea pigs received 10 intracutaneous applications of the test material over 3 wk induction, Freund's complete adjuvant (1:1) incorporated in the second week of induction; after 2-wk rest, guinea pigs were challenged separately via intradermal and occlusive epidermal treatments	All animals in all strains had positive reactions to the test material	[53]
p-Phenylenediamine; purity not reported	induction: distilled water (epidermal) and saline (intradermal) challenge: distilled water	intradermal induction: 0.1% dermal induction: 0.5% challenge: 1%	5 male albino guinea pigs/ group	Guinea pig sensitization study; for induction, 5 guinea pigs received the test material dermally on abraded skin and 5 guinea pigs received the test material as intradermal injections over a 2-wk period; after a 2_wk rest period, animals received 1% challenge on intact skin; sites scored after 24 h	Sensitizing in 4/5 animals induced intradermally; none of the animals induced dermally were sensitized	[5]
p-Phenylenediamine; purity not reported	induction: propylene glycol challenge: 100% alcohol	induction: 1% challenge: 5%	10 male albino guinea pigs, additional group of 10 unexposed animals were control	Guinea pig sensitization study; animals received 1 intradermal injection of 1% solution for induction; after 3-wk rest, animals challenged with 5% solution to intact and abraded skin; 10 untreated animals served as controls; sites scored after 24 h	Sensitizing; 8/10 animals had either moderate erythema, strong erythema, or erythema with edema	[5]

Table 9. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
p-Phenylenediamine; purity not reported	dimethyl phthalate	induction: 1% challenge:2.5 and 25%	groups of 10 male Dunkin-Hartley guinea pigs	Intracutaneous sensitization study; induction phase consisted of 4 sacral intradermal injections (1 injection/wk); after 2-wk rest, animals challenged by applying test material on separate shaved intact sites, responses scored 24 and 48 h post-application; additional group of previously unexposed animals also received the same challenge	Mild sensitizer; at challenge, 4/10 animals had sensitization response to 25% test material, 3/10 animals that were not induced had mild erythema to 25% test material	[5]
p-Phenylenediamine; purity not reported	acetone:dimethyl phthalate (1:9)	induction: 1% challenge: 3 and 30%	10 male Dunkin-Hartley guinea pigs	Intracutaneous sensitization study, as above, except an unexposed group was not used	Sensitizing; moderate erythema to erythema and edema observed during induction with some animals exhibiting blanching and necrotic centers; during challenge, animals exhibited mild erythema and erythema with edema at the 30% concentration sites, 10/10 animals had significant sensitization score increases during challenge phase	[5]
p-Phenylenediamine; purity not reported	induction: saline challenge: distilled water	induction: 1% challenge: 3 and 30%	groups of 10 male Dunkin-Hartley guinea pigs	Intracutaneous sensitization study, as above.	Mild sensitizer; at challenge, 4/10 animals had sensitization response to 30% test material, no sensitization was observed in the animals that were not induced	[5]
p-Phenylenediamine; 100% pure	induction: dimethyl phthalate challenge: 13% guinea pig fat in 50/50 acetone/dimethyl ethane	induction: 1% challenge: 5 and 10%	10 male albino guinea pigs; a group of 5 additional animals for non-induced control	Intracutaneous sensitization study, as above.	Moderate sensitizer; 6/10 guinea pigs had moderate to strong sensitization response (erythema) to 10% test material at 48 h reading; no sensitization was observed in the animals that were not induced at 48 h	[5]
p-Phenylenediamine; purity not reported	induction: dimethyl phthalate challenge: 13% guinea pig fat in 50/50 acetone/dimethyl ethane	induction: 1% challenge: 10 and 25%	10 male albino guinea pigs; a group of 5 additional animals for non-induced control	Intracutaneous sensitization study, as above.	Sensitizing; 8/10 guinea pigs had mild erythema at 48 h reading; no reactions observed in negative controls	[5]
p-Phenylenediamine; purity not reported	induction: dimethyl phthalate challenge: 13% guinea pig fat in 50/50 acetone/dimethyl ethane	induction: 1% challenge: 10 and 25%	10 male albino guinea pigs, additional group of 10 unexposed animals were control	Intracutaneous sensitization study, as above. Additionally, after challenge, sutures were implanted in the 10 sensitized guinea pigs and were rechallenged 4 wk later	7/10 animals sensitized in initial challenge and 6/10 animals sensitized at rechallenge 4 wk after implantation of sutures; no sensitization observed in control animals	[5]
p-Phenylenediamine; 100% pure	induction: dimethyl phthalate challenge: 13% guinea pig fat in 50/50 acetone/ dimethyl ethane	induction: 1% challenge:1%	10 male albino guinea pigs; a group of 5 additional animals for non-induced control	Intracutaneous sensitization study (Terhaar procedure); induction phase consisted of injecting 1% test material in whole rabbit blood in rear foot pad; after 2-wk rest, animals challenged by applying 1% test material on shaved intact sites, responses scored 3 and 24 h post-application; additional group of 5 previously unexposed animals also received the same challenge	Mild sensitizer; 2/10 guinea pigs had moderate sensitization response (erythema) to 1% test material at 24 h reading; no sensitization was observed in the animals that were not induced at 24 h	[5]

Table 9. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
			HUMAN			
p-Phenylenediamine; purity not reported	pet.	1%	98 healthy subjects	HRIPT; patches applied on upper out arm for 5 min 3 times a wk for 3 wk; challenge left in place for 48 h and read 30 min and 48 h after removal; occluded patches were 2 cm^2	1 subject was determined to be pre-sensitized to test material, 3 subjects were sensitized, 2 subjects had irritant reactions	[54]
p-Phenylenediamine; purity not reported	formulation	0.96% (on head 0.48%) in Group 1; 3% in Group 2; 0% in Group 3 (controls)	Group 1 had 1107 subjects; Group 2 had 548 subjects; Group 3 had 516 subjects	6 mo in-use study; all panelists pre-screened for *p*-Phenylenediamine allergy with a 48-h patch test at 1% in pet.; subjects divided into 3 groups, Group 1 used hair dye containing 0.96% *p*-Phenylenediamine 5 min/d for first 4 d and then 5 min/d once/wk; Group 2 used hair dye containing 3% *p*-Phenylenediamine 30-40 min once/mo for a total of 6 exposures; Group 3 were unexposed; at study end and after a 3- to 4-wk rest, all panelists retested with 1% *p*-Phenylenediamine pet. in a 48-h patch test along with an open test	In pre-screen, 69 of 2545 subjects had a positive reaction to the test material at 1% and were excluded from the study; following the 1% occluded patch test at study end, 7.2, 1.3, and 0.4% of subjects from Group 1, Group 2, and Group 3, respectively, had positive reactions to *p*-Phenylenediamine; almost all reactions observed were grade 1; in the open test for all groups, 1/3 subjects that tested positive in the occluded test were positive in the open test. The authors noted that reduction in the exposure duration from 48 h to 5 min decreased the rate of sensitization from 54% to 3%. However, infrequent but longer duration and higher concentration of exposure to *p*-Phenylenediamine was significantly less likely to induce sensitization compared to more frequent, shorter duration, and lower concentration exposure.	[54]

Table 10. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
ANIMAL						
p-Phenylenediamine; purity not reported	in formulation	5, 10 or 15%	groups of 10 rats; no further details provided	test material instilled into 1 eye once daily for up to 3 mo; no further details provided	Keratitis and corneal opacities observed; no further details provided	[5]
p-Phenylenediamine; purity not reported	not reported	2.5% w/v	guinea pigs; no further details provided	ocular irritation study; observations made 0.5, 1, 2, 3, 4, 6, 7, and 24 h after treatment; no further details provided	Not irritating	[5]
p-Phenylenediamine; purity not reported	not reported	2.5% solution containing 0.05% sodium sulfite	3 rabbits; details not provided	test material instilled in rabbit eye and then rinsed with water after 10 s	Minimal conjunctival irritation observed in only 1 animal	[7]
p-Phenylenediamine; purity not reported	not reported	5% and in 4 formulations at unknown concentrations	rabbits; details not provided	details not provided	Test material at 5% considered a weak irritant; no irritation observed in the 4 formulations containing *p*-Phenylenediamine; no further details provided	[5]
p-Phenylenediamine; 99.5% pure	no vehicle	neat	2 male albino rabbits	ocular irritation study in accordance with OECD TG 405; 10 mg place in right conjunctival sac of each rabbit; afer 20 s, 1 treated eye was washed with tap water for 1 min; the treated eye of the other rabbit was not washed; observations made 1 and 4 h and 1, 2, 3, 7, and 14 d after treatment	Moderately irritating; in unwashed treated eye, generalized slight corneal cloudiness, moderate iritis, and moderate conjunctivitis observed; in washed treated eye, generalized slight corneal cloudiness, moderate iritis, and mild conjunctivitis observed; both treated eyes were normal within 14 d	[5]
p-Phenylenediamine; purity not reported	not reported	not reported	rabbits; no further details provided	ocular irritation study; dry powder and saturated solution used; no further details provided	Dry test material produced immediate signs of discomfort, lacrimation, blepharospasm, and vascular conjunctival inflammatory reaction resembling conjunctivitis, with promptly increasing palpebral edema; no persistent effects observed in cornea or conjunctival membrane, even after repeated applications; saturated solution had no immediate sensory reactions, but vascular conjunctival reaction was observed immediately after instillation, increased for a few min, and then rapidly cleared	[5]

Table 11. Multicenter and retrospective studies

# Patients	Clinical Testing Type	Location	Years	Results	Reference
		Multicenter Studies			
1138	Multicenter study of patients with suspected allergic contact dermatitis; 1% *p*-Phenylenediamine in petrolatum tested using Finn chambers or A1 test patches for 48 or 72 h	North America	January 1, 1984 to May 1, 1985	79 allergic reactions and 2 irritant reactions reported; relevance value of 59% assigned to *p*-Phenylenediamine	[63]
3515	Multicenter study of patients tested at NACDG clinics using standardized patch testing technique with 52 allergens that included 1% *p*-Phenylenediamine	North America	July 1, 1992 to June 30, 1994	6.3% (527) patients had positive reaction to *p*-Phenylenediamine	[62]
3111	Multicenter study of patients tested at NACDG clinic using standardized patch testing technique with 49 allergens that included 1% *p*-Phenylenediamine pet.	North America	July 1, 1994 to June 30, 1996	6.8% of patients had positive reaction to *p*-Phenylenediamine	[60]
5831	Multicenter study of patients tested at NACDG clinic using standardized patch testing technique with 50 allergens that included 1% *p*-Phenylenediamine pet.	North America	July 1, 1998 to December 31, 2000	4.9% of patients had positive reaction to *p*-Phenylenediamine	[61]
36,491	Multicenter study of patients tested at IVDK with standard series, including 1% *p*-Phenylenediamine pet.	Germany and Austria	1990 to 1995	5.0% (crude rate, 4.8% standardized for age and sex) had positive reactions to *p*-Phenylenediamine	[64]
1141	Population-based nested, case-control study; patch tests with 25 standard allergens as recommended by the ICDRG and the German Contact Dermatitis Group that included 1% *p*-Phenylenediamine	Germany	between 1994 and 1995	1.5% of patients overall had positive reaction to *p*-Phenylene-diamine	[66]
2034	Multicenter study of female consumers at IVDK in whom hair cosmetics were suspected as cause of contact dermatitis; German Contact Dermatitis Research Group hairdressers series patch test including 1% p-Phenylenediamine pet.	Germany, Austria, and Switzerland	January 2013 to December 2020	31.6% (580 reactions) of consumers had positive reactions to p-Phenylenediamine	[92]
		Retrospective Studies			
54,917	Retrospective study of individuals tested to 1.0% *p*-Phenylenediamine pet. by the NACDG	North America	1994 to 2018	5.6% (3095) of patients had positive reaction to *p*-Phenylene-diamine; over half (55.3%) of reactions were ++ or +++ at final reading and 60.9% were relevant	[69]
36,064	Retrospective study of patients with suspected allergic contact dermatitis that returned for delayed readings between days 7 and 10 or beyond; patch testing was performed by the Mayo Clinic and included 1% p-Phenylenediamine	United States	October 1997 to December 2006	Out of 251 positive reactions, only 1 patient was negative on day 5 of testing but positive at or after day 7; most reactions (241) resolved before day 5	[73]
38,775	Retrospective study of NACDG patch test data associated with hair care products; screening series patches of 65-70 allergens including 1% *p*-Phenylenediamine pet.	North America	2001 to 2016	35.8% (1524) of patients had reaction to *p*-Phenylenediamine	[67]
3088	Retrospective study of patients patch tested with Mayo clinic standard series; results compared to patch testing results from 2001 to 2005; *p*-Phenylenediamine was tested at 1% in pet.	United States	2006 to 2010	5.2% of patients had positive reaction to *p*-Phenylenediamine; in 2001 to 2005, 4.5% of 3832 patients were positive	[77]
2313	Retrospective study of patients patch tested with the standard series at Massachusetts General Hospital, including 1% *p*-Phenylenediamine pet.; results were compared to testing performed by same hospital between 1998 to 2006 and 1990 to 2006	United States	January 2007 to December 2016	4.0% of patients had positive reaction to *p*-Phenylenediamine; the rate of positivity was 3.1% in patients tested both between 1998 to 2006 (n = 627) and 1990 to 2006 (n = 1237)	[80]
2568	Retrospective study of patch test reactions with the Mayo clinic standard series allergens and compared to results to earlier NACDG reports; 1% *p*-Phenylenediamine pet.	North America	2011 to 2015	4.4% (114 reactions) of patients had positive patch test reactions to *p*-Phenylenediamine; reactions for 2011 to 2012 were 6.3% of 4223 patients tested; for 2012-2014, 7.0% of 4853 patients tested	[75]
149	Retrospective study of black patients with allergic contact dermatitis; testing was performed with the Mayo clinic standard, extended standard, or hairdresser series, including 1% p-Phenylenediamine pet.	United States	January 2011 to December 2020	6.1% (9 reactions) of patients had positive patch test reactions to p-Phenylenediamine	[74]
60	Retrospective study of patients with frontal fibrosing alopecia and lichen planopilaris to determine connection with allergic contact dermatitis; patients tested with the North American baseline and cosmetic and hairdressers series, including *p*-Phenylenediamine (concentration and vehicle not described)	United States	2015 to 2022	10% (6 reactions) of patients had positive reactions to *p*-Phenylenediamine	[94]
2686	Retrospective study of patients patch tested with Mayo clinic standard series; *p*-Phenylenediamine was tested at 1% in pet.	United States	2017 to 2021	5.2% (141 reactions) of patients had positive reaction to *p*-Phenylenediamine	[76]
4107	Retrospective study of patients with allergic contact dermatitis using NACDG screening series and supplemental allergens as needed, *p*-Phenylenediamine tested at 1% in pet.	North America	January 2019 to December 2020	5.6% (231 reactions) of patients had positive reactions to *p*-Phenylenediamine	[68]

Table 11. Multicenter and retrospective studies

# Patients	Clinical Testing Type	Location	Years	Results	Reference
500 children	Retrospective study to determine whether the site of primary dermatitis in children could predict an allergic contact dermatitis diagnosis; age group ranged from 0-15 y; British Contact Dermatitis standard series including 1% *p*-Phenylenediamine in petrolatum; Finn chambers for 48 h	Leeds, United Kingdom	between 1995 and 2004	8% (11 reactions) of patients had positive reaction to *p*-Phenylenediamine; allergy found only in children over 5 yr; henna tattoos most common source of *p*-Phenylenediamine allergy in children aged 5-10 yr and hair dye in older children	[70]
156	Retrospective study with extended British standard series in addition to supplementary series and patients' own products where relevant; 48 h occluded tests with polyethylene plastic chambers; 1.0% *p*-Phenylenediamine pet.	London, UK	October 2016 to April 2018	8.3% (13 reactions) of patients had positive reaction to *p*-Phenylenediamine	[95]
826	Study of late patch test reactions of at least 10 d after application; ICDRG test guidelines using Finn chambers; 1% *p*-Phenylenediamine pet.	Finland	January 2002 to February 2006	3.1% (26 reactions) of patients had positive reaction to *p*-Phenylenediamine, with late reactions observed in 6 patients (0.75% of 826)	[78]
200	Retrospective study on patients with rosacea that underwent patch testing with the standard series of the Spanish Contact Dermatitis and Skin Allergy research group and additional series as needed; testing performed with Finn chambers for 48 h and included *p*-Phenylenediamine (concentration and vehicle not reported)	Valencia, Spain;	May 1991 to May 2019	5.5% (11) of patients had positive reactions to *p*-Phenylene-diamine	[65]
9341	Retrospective study of patients with contact allergy; testing performed with standard series of the Spanish Research Group on Contact Dermatitis and Skin Allergies and included 1% *p*-Phenylenediamine pet.	Spain	2004 to 2014	4.1% (386 reactions) of patients had positive reaction to *p*-Phenylenediamine	[79]
501	Retrospective study of patients with suspected allergic contact dermatitis to hair dyes; patients tested with European baseline series and an extended hairdressing series, *p*-Phenylenediamine was tested at 1%	Greece	2010 to 2019	52.5% (189 reactions) of patients had positive test reactions to *p*-Phenylenediamine; 126/226 customers and 63/136 hairdressers had positive reactions	[89]
251 children and adolescents	Retrospective study of children ≤ 18 years of age with suspicion of allergic contact dermatitis; patch tests performed with the extended European baseline series and/or additional series including hairdresser serries or cosmetic series; 1% *p*-Phenylenediamine pet. only tested if allergic contact dermatitis was suspected to be from black henna tattoos or hair dye; 0.5% pet. was used in 4 patients with severe reactions (open test technique in 2)	Turkey	1996 to 2017	8.4% (21 reactions) of patients had positive reactions to *p*-Phenylenediamine; reactions observed only in adolescents (ages 10 – 18 yr), reactions were reported to hair dye (n = 16), black henna (n = 3)	[71]
1309	Retrospective study of patch test results of patients with suspected contact allergies; European baseline series, included 1% *p*-Phenylenediamine pet.	Turkey	2013 to 2019	3.7% (48 reactions) of patients had positive reactions to *p*-Phenylenediamine	[82]
191 children	Retrospective study of pediatric patients diagnosed with allergic contact dermatitis; European baseline patch test series including *p*-Phenylenediamine (concentration and vehicle not reported)	Turkey	2015 to 2019	2.61% (5 reactions) of patients had positive reaction to *p*-Phenylenediamine	[93]
152	Retrospective study of patients with clinically suspected allergic contact dermatitis; patients tested with thin-layer rapid-use epicutaneous (TRUE) patch tests including p-Phenylenediamine (no further detail provided on testing)	Saudi Arabia	January 2012 to February 2015	22.9% (17 reactions) of patients had positive reactions to *p*-Phenylenediamine	[90]
101	Retrospective study of patients with dermatitis on photo-exposed body areas suspected of being chronic actinic dermatitis; patch testing using Indian standard series containing 1% *p*-Phenylenediamine pet done in all patients and photo-patch test using Scandinavian photo-patch antigen series performed in 86 patients	India	2010 to 2014	5% (5 reactions) of patients had positive patch test reactions to *p*-Phenylenediamine	[88]
106	Retrospective study of patients with pigmented cosmetic dermatitis and allergic contact dermatitis; patients patch tested with Indian cosmetic and fragrance series, relevant allergens from Indian standard series, extended hairdressing series in cases of suspected contact dermatitis to dyes or had positive patch test to *p*-Phenylenediamine, and patients' cosmetic products used prior to onset of dermatitis; 1% *p*-Phenylenediamine pet. tested	New Delhi, India	January 2015 to October 2017	25% (27 reactions) of patients had positive reaction to *p*-Phenylenediamine, this ingredient was predominately associated with allergic contact dermatitis ($p < 0.001$) and not pigmented cosmetic dermatitis	[87]
152	Retrospective study of patients with chronic palmoplantar vesicular dermatitis; testing performed with Indian Standard Battery (included 1% *p*-Phenylenediamine) and patients' own materials; ICDRG test guidelines used to grade sites	India	dates not reported	9.2% (14 reactions) of patients had positive reaction to *p*-Phenylenediamine	[85]

Table 11. Multicenter and retrospective studies

# Patients	Clinical Testing Type	Location	Years	Results	Reference
80	Retrospective study of 80 consecutive patients with suspected hair dye allergy; testing performed with Indian standard patch test series and included 1% *p*-Phenylenediamine pet.; ICDRG test guidelines used to grade sites	India	dates not reported	67.5% (54 reactions) of patients had positive reaction to *p*-Phenylenediamine, another 3 patients had irritant reactions	[86]
438	Retrospective analysis of contact dermatitis patients; testing performed with European baseline series or Shoe series, both including *p*-Phenylenediamine (no detail provided on concentration or vehicle)	Sri Lanka	2012 to 2018	12.3% (54 reactions) of patients had positive reactions to *p*-Phenylenediamine	[91]
2842	Retrospective study to study incidence of patch test reactions to hair cosmetic allergens; baseline patch series with modification of the European and International baseline series in addition to the hairdressing series; testing performed with Finn chambers for 48 h and included 1.0% *p*-Phenylenediamine pet.	Thailand	2009 to 2018	6.4% (182) of patients had positive reactions to *p*-Phenylene-diamine	[84]
4903	Retrospective study of patients with suspected allergic contact dermatitis; patch testing with modified European standard series and other allergens, including 1% *p*-Phenylenediamine pet.	Singapore	November 2007 to October 2017	13.4% (399) of patients had positive reactions to *p*-Phenylene-diamine	[83]
5865	Retrospective study of patients patch tested with the Japanese baseline series, including *p*-Phenylenediamine	Japan	April 2015 to March 2019	8.9% of patients had positive reactions to *p*-Phenylenediamine	[81]
2402	Retrospective study for the development of the New Zealand baseline patch test series from 4 patch test clinics; 1% *p*-Phenylenediamine pet. was included in the series	New Zealand	2008 to 2020	4.9% of patients had positive reaction to *p*-Phenylenediamine	[72]

Table 12. Case reports related to hair dye use

Patient(s)	Presentation	Patch Test Results	Reference
8 Arabic men	Beard dermatitis following use of hair dye to facial hair; lesions were pruritic, erythematous, popular eruptions in the jaw area after each dye application, onset ranged from 24- to 48-h after dye application	Patch tests were positive for *p*-Phenylenediamine	[96]
38-yr-old male	Swelling of the neck with no pain or itching 3 d after dying beard; no allergies or previous history of present symptoms; "band-like" maculopapular rash with edema observed across mid neck over laryngeal prominence and 1 in below beard line; beard dye contained *p*-Phenylenediamine	Patient not tested	[101]
29-yr-old female	Cough along with pruritic eruptions on the scalp and neck several hours after using 2 kinds of hair dye on the same day, cough worsened with dyspnea appearing after 4 d; patient had a 2 yr history of hair dye-induced dermatitis, allergic rhinitis, and asthma	Open test and scratch test were performed using 1% *p*-Phenylenediamine pet., hair dye 1 (containing *p*-toluenediamine), and hair dye 2 (containing *p*-Phenylenediamine), no wheals or erythema were observed after 15 min; subsequently, scratch test material were removed while the open test materials were allowed to dry; a closed patch with 1% *p*-Phenylenediamine was also performed; after 5 h, pruritus appears at the sites of the open and closed tests; after 16 h, patient developed hoarseness, pharyngeal symptoms, and dyspnea; on day 2 and day 3, open test was strongly positive for *p*-Phenylene-diamine and dye 1 color solution and the 16-h closed patch test was strongly positive to *p*-Phenylene-diamine; the 15-min scratch test yield positive reactions at 16 h, day 2, and day 3 to the color solutions of hair dyes 1 and 2	[97]
50-yr-old male	Swelling of both eyelids 8 h after use of hair dye; face and lips also became swollen and itchy, exudative lesion developed on the scalp	Positive reaction (+++) to hair dye and to *p*-Phenylene-diamine in Indian standard series and cosmetics and fragrance series	[99]
27-yr-old female	Severe edema involving upper and lower eyelids of both eyes, forehead, and scalp; initially diagnosed with angioedema; thorough systemic examination revealed no other focus of allergic activity and patient had no other history of atopic event of allergic reactions; patient had used hair dye for first time ever 2 d before reaction; 2 yr prior patient had a black henna tattoo without reaction	Positive reaction (papules and vesicles on erythematous test area) to *p*-Phenylenediamine in European standard series	[100]
8 children aged 12 to 15 yr	Edema and eczema of varying degree to the ears, forehead, eyes, neck, and/or face following hair dye use; 6/8 report previous exposure with reaction to black henna tattoo; 5/8 required hospitalization	All patients had ++ or +++ reactions to 1% *p*-Phenylenediamine in petrolatum except one that was ++ at 0.1%; simultaneous positive patch reactions observed to IPPD and local anesthetics	[102]
61-yr-old male	Pruritic eruptions on upper back; several months prior, patient had recurring pruritic sensation of scalp and pruritic eruptions on the calp and upper back following use of hair dye; patient had used the hair dye for 12 yr	Patient was + to 1% *p*-Phenylenediamine pet for up to 38 d after patch test initiated	[104]
74-yr-old female	Erythematous, scaly and pruritic rash of scalp with associated hair loss and erythematous popular rash on lumbar area for 14 mo; 18 mo prior, patient underwent radiotherapy for ductal carcinoma of the breast, symptoms started after 2nd treatment session; patient had dyed hair for 20 yr prior without incidence	Patch test was +++ to *p*-Phenylenediamine, ++ to methylchloroisothiazolinone, and + to carba mix and gold	[105]

Table 13. Case reports related to tattoo use

Patient(s)	Presentation	Patch Test Results	Reference
26-yr-old female	Hypertrophic allergic contact dermatitis following application of black hair dye to skin	0.5% *p*-Phenylenediamine pet. using Finn chambers; scored on days 2 and 3; papulovesicular reaction observed	[130]
3 female patients aged 32, 23, and 25 yr	Blistering eruptions on dorsum of hands, forearms, and/or feet within a week or 2 after application of black hair dye to skin	1% *p*-Phenylenediamine pet. in standard and hairdressers series; positive reactions ranging from 1+ to 2+ on day 2 to 2+ to 3+ on day 4	[131]
4 patients aged 7, 8, 20, and 25 yr old	Contact dermatitis on skin painted with black henna	1% *p*-Phenylenediamine pet. with Finn Chambers for 48 h; scored at 48 and 72 h; 3 out of 4 patients positive for *p*-Phenylenediamine	[115]
3 patients aged 10 (female), 17 (male), and 8 (female) yr	Allergic contact dermatitis at site of henna dye application (arm and neck)	3+ reaction to *p*-Phenylenediamine	[132]
4 patients aged 31, 32, 33, and 43 yr old	Itching, erythema, and swelling at the site of black henna tattoo application, 2- to 10-d post-application; microscopic examination revealed spongiotic dermatitis with dense lymphohistiocytic infiltrates	Strongly positive reactions to *p*-Phenylenediamine in pet. at 72 h	[120]
37-yr-old female	Pruritic dermatitis on left upper arm and lower back within 24 to 48 h of application of black henna tattoo	1% *p*-Phenylenediamine in pet. using Finn chambers; after 7 h, test sites became severely pruritic; after 24 h, 3+ reaction was observed at both test sites; by 1 wk, reaction persisted and remained strongly positive; no reactions in 10 control patients	[125]
10 patients aged 18 to 28 yr old	Inflamed skin eruptions after receiving temporary paint-on tattoos	6 patients patch tested with 48-h IQ chamber; all had moderately to strongly positive reactions to *p*-Phenylene-diamine after 72 h	[119]
6 patients (3 male, 3 female) ranging in age from 11 to 18 yr	Allergic contact dermatitis following skin painting with black henna	2+ to 3+ reactions to *p*-Phenylenediamine	[124]
11-yr-old male	Burning sensation and marked redness at site of tattoo application in right brachium that evolved into pronounced redness and scaling after 10 d; visible residual hypopigmentation observed 4 wk after tattoo application; patient had received a temporary tattoo 1 yr prior	Positive patch results to 0.5% *p*-Phenylenediamine	[122]
15-yr-old male	Erythematous and edematous reaction, including pruritis and pain, on left arm that occurred within 48 h of applying a black henna tattoo; cutaneous examination showed well-demarcated, indurated, erythematous papulovesicular eruption within the borders of the tattoo on the flexural site of the left arm	3+ reaction to *p*-Phenylenediamine in European standard series hat was evaluated after 48, 72, and 96 h; negative reaction to pure henna	[123]
22-yr-old male	Itchy, slightly painful, and bullous, keloidal eruption at the site of a henna tattoo on left forearm	3+ reaction to *p*-Phenylenediamine after patch test with henna powder, *p*-Phenylenediamine, and European standard series; patch with pure henna powder and in alcohol solution and with other allergens negative	[127]
11-yr-old male	Sharply demarcated, livedoid-colored, pruritic scorpion-shaped plaque containing many vesicles, bullae, and yellowish crust on left forearm, with satellite papules and papulovesicles around lesion, the trunk, and the face; reaction occurred within 24 h of receiving a black henna tattoo; patient had previously received a temporary tattoo on right shoulder and developed a mild eczematous reaction within 2 wk of application	Potent reaction to *p*-Phenylenediamine at 48 and 96 h following European standard series patch test	[116]

Table 14. Occupational exposure studies

Occupation	Study methods	Study results	Reference
302 hairdressers, 43 males and 259 females	Multicenter study to evaluate the frequency and source of contact sensitization in hairdressers with dermatitis; patients tested with Gruppo Italiano Ricerca Dermatiti da Contatto e Ambientali (GIRDCA) standard series using Finn chambers; *p*-Phenylenediamine was tested at 1% in pet. and the hydrochloride salt was tested at 0.5% in pet.	Frequency of 16.6% (50 reactions) recorded for *p*-Phenylenediamine and 7.6% (23 reactions) recorded for the hydrochloride salt; cross-reactions to benzocaine, diaminodiphenylmethane, and *para*-derivatives	[149]
128 hairdressers, 125 males and 3 females	Hairdressers exposed to oxidative hair dyes under controlled conditions for 6 work days; subjects colored hairdresser training heads (manikins) with 2% [^{14}C]*p*-Phenylenediamine for 6 h/d; gloves were worn during the application and rinsing of the dye to the heads; urine and blood analysis performed on all subjects	Adverse events not reported in any subjects; hair + scalp accounted for 53.46 ± 4.06% of the applied radioactivity; concentration of dye in the plasma was below the limit of quantification (≤ 10 ng *p*-Phenylenediamine$_{eq}$/ml); total urinary excretion of radiolabel ranged from a total of < 2 to 18 µg *p*-Phenylenediamine$_{eq}$, and was similar in subjects exposed during the different phases of hair dyeing; mean mass balance of radiolabel for the 6 d study was 102.50 ± 2.20%; overall mean total systemic exposure of hairdressers to oxidative hair dyes during a work day that included 6 hair dyeing processes was estimated to be < 0.36 µg *p*-Phenylenediamine$_{eq}$/kg bw/work d	[150]
355 female hairdressers	Study of the occurrence and cause of hairdressers' occupational skin and respiratory diseases; 130 with work-related skin and respiratory symptoms underwent physical examinations, lung function tests, prick and patch testing, and nasal and lung provocation tests; 48-h patch testing with the European standard series and the hairdresser series	2 out of 54 subjects that underwent patch testing were positive to *p*-Phenylenediamine	[152]
33 hairdressers, 13 males and 20 females	Hand rinse study to assess skin exposure to permanent hair dye compound in hairdressers; samples collected from each hand before the start of hair dyeing, after application of the dye, and after cutting newly-dyed hair; 16 hairdressers did not use gloves during application of the dye and none wore gloves for cutting hair; samples analyzed for pertinent aromatic amines and resorcinol using HPLC; 10 of 54 hair dyes contained *p*-Phenylenediamine	After the application step, *p*-Phenylenediamine found in samples from 4 hairdressers, 3 of which had used gloves during application of the dye; after the cutting step, *p*-Phenylenediamine was found in the samples from 5 hairdressers; hairdressers' skin is found to be exposed to allergenic compounds during hair dyeing, with exposure occurring during application, cutting, and background; exposure loading for *p*-Phenylenediamine was 22 - 989 nmol/hand	[153]
324 hairdressers	Investigation of the characteristics and incidence of contact dermatitis among hairdressers in northeastern Italy between 1996 and 2016; patch testing with European baseline series, Trivenento extended series, and hairdresser series using Finn chambers, included 1% *p*-Phenylenediamine; 9660 matched controls	66 out of 324 (20.4%) hairdressers positive to *p*-Phenylenediamine, 322 out of 9660 (3.3% controls positive	[154]
54,917 patients	Retrospective study of individuals tested to 1.0% *p*-Phenylene-diamine pet. by the NACDG between 1994 to 2018	Out of 3095 positive reactions, 8.3% (237) were occupationally related, with the most common of these (72.8%) occurring in hairdressers/cosmetologists	[69]
72 hairdressers	A cross-sectional study of professional exposure to *p*-Phenylenediamine (median exposure = 6 yr) in henna mixed dye, which ranged in concentration from 10% (in formulation) to 97% (pure form) *p*-Phenyulenediamine; subjects were from 6 salons in Sudan; patients were evaluated for presence of renal impairment (serum creatinine ≥ mg/dl) and other markers of kidney damage	Renal impairment, proteinuria, and hematuria were observed in 14, 26.4, and 41.1% of the hairdressers, respectively; hypertension, skin changes, and bronchospasm were found in 19.4, 38.9, and 22% of hairdressers, respectively; using the high concentration (pure form), *p*-Phenylenediamine significantly increased the risk of having elevated serum creatinine ((OR 5.9; p = 0.02) and proteinuria (OR 9.8; p = 0.002) compared to the ingredient in formulation	[155]
787 hairdressers	Multicenter study of female hairdressers at IVDK between 2013 to 2020; German Contact Dermatitis Research Group hairdressers series patch test including 1% *p*-Phenylene-diamine pet.	156 out of 787 (19.7%) hairdressers positive to *p*-Phenylenediamine	[92]
46 farmers, 21 males and 25 females	Study to determine if dermatitis in farmers was secondary to an occupational allergen; patients tested with European standard, fragrance, antimicrobial and preservative, and an agricultural series using Finn chambers; *p*-Phenylenediamine tested at 1%; a *p*-Phenylenediamine rubber mix was tested at 0.6%	2 reactions observed to *p*-Phenylenediamine and 2 reactions observed to *p*-Phenylenediamine rubber mix (4 farmers total); all initially presented with hand dermatitis	[151]

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