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

Status:	Draft Final Amended Report for Panel Review
Release Date:	May 10, 2024
Panel Meeting Date:	June 3-4, 2024

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Christina Burnett, M.S., Senior Scientific Analyst/Writer.

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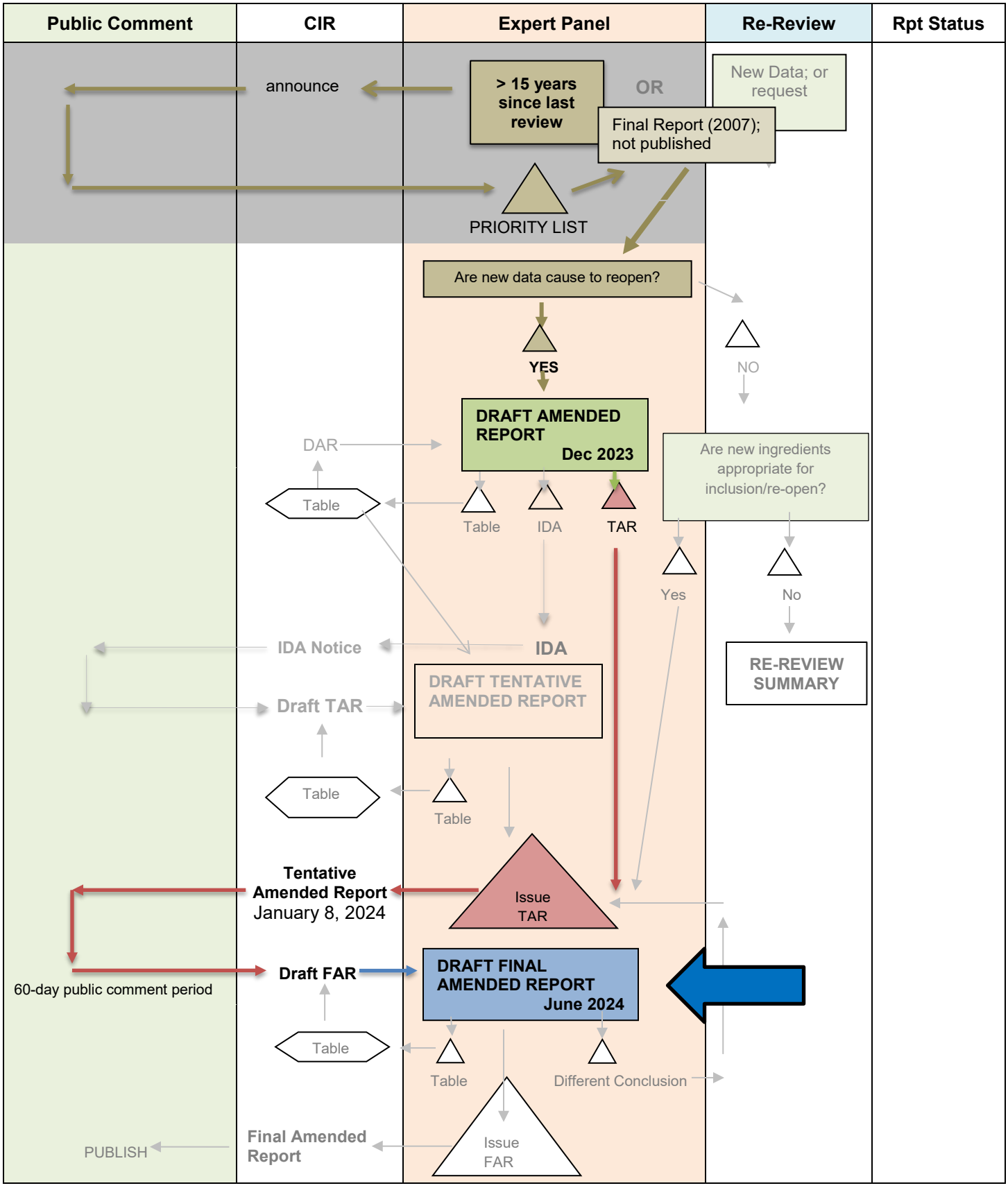
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY *p*-Phenylenediamine ingredients

MEETING June 2024



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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, M.S., Senior Scientific Analyst/Writer, CIR
Date: May 10, 2024
Subject: Amended Safety Assessment of *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate as Used in Cosmetics

Enclosed is the Draft Final Amended Report on the Safety Assessment of *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate as Used in Cosmetics. (It is identified as *report_Phenylenediamine_062024* in the pdf document.) At the December 2023 meeting, the Panel issued a Tentative Amended Report with the conclusion that *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate are safe for use as hair dye ingredients in the present practice of use and concentration described in the safety assessment.

Since the December meeting, no unpublished data have been received for this report. However, additional published case studies and an in vitro cytotoxicity study have been added to the safety assessment and are highlighted to aid in the Panel's review. Comments provided by the Council on the Tentative Amended Report have been addressed (*PCPCcomments_Phenylenediamine_062024* and *response-PCPCcomments_Phenylenediamine_062024*). Additionally, comments provided by the Women's Voice for the Earth on the Tentative Amended Report and a response addressing these comments are also included in this report package (*WVEcomments_Phenylenediamine_062024* and *response-WVEcomments_Phenylenediamine_062024*).

Other supporting documents for this report package include a flow chart (*flow_Phenylenediamine_062024*), the original report (*originalreport_Phenylenediamine_062024*), the initial 2006 re-review summary (*rereview2006_Phenylenediamine_062024*), the 2007 Final Amended Report (*amendedreport2007_Phenylenediamine_062024*), report history (*history_Phenylenediamine_062024*), a search strategy (*search_Phenylenediamine_062024*), minutes from all the meetings at which *p*-Phenylenediamine and its salts were discussed during the original reviews (*originalminutes_Phenylenediamine_122023*), transcripts from the meeting at which this amended report was discussed (*transcripts_Phenylenediamine_062024*), and a data profile (*dataprofile_Phenylenediamine_062024*).

The Panel should carefully review the Abstract, Discussion, and Conclusion, and issue a Final Amended Report.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: January 23, 2023

SUBJECT: Tentative Amended Report: Safety Assessment of p-Phenylenediamine, p-Phenylenediamine HCl and p-Phenylenediamine Sulfate as Used in Cosmetics (release date: January 8, 2024)

The Personal Care Products Council respectfully submits the following comments on the tentative amended report, Safety Assessment of p-Phenylenediamine, p-Phenylenediamine HCl and p-Phenylenediamine Sulfate as Used in Cosmetics.

Key Issues

ADME, Human Dermal; Table 14 – There are two studies in Table 14 regarding exposure and ADME in hairdressers. These studies (references 150 and 153) should be presented in the ADME section. The study in 128 hairdressers (reference 150) is a study that provides measures of exposure used to estimate daily exposure (<0.36 µg p-Phenylenediamine$_{eq}$/kg/bw/work day) in hairdressers that completed 6 hair dying processes in a day.

Ocular Irritation; Summary; Table 10 – The ECHA dossier rated the 3-month ocular test as not reliable. Please consider removing this study from the CIR report. In addition, the ECHA dossier says that the test substance was a hair dye containing p-Phenylenediamine. The dossier does not say the concentration of p-Phenylenediamine in the hair dye. The dossier says that 5, 10, or 15% of the test substance was instilled into the eyes of rats daily for up to 3 months, in contrast to the CIR report which incorrectly states that "rats received up to 15% p-Phenylenediamine in formulation" – the concentration of p-Phenylenediamine is not stated in the dossier. The concentration(s) of hair dye resulting in keratitis and corneal opacities was not clearly stated in the ECHA dossier, while the text of the CIR report suggests that it was at 15%. If this study is left in the CIR report, the description should be corrected so that it is consistent with the information in the ECHA dossier.

Multicenter and Retrospective Studies; Summary; Table 11 – Please review the studies in Table 11 again. Studies with the very high rates of sensitization, such as 67.5% in patients from India, were studies of patients with suspected hair dye allergy, or suspected hair care product allergy. It

would be helpful if this was stated in the text. In Table 11, it would be helpful to identify the patient selection criteria in both the Clinical Testing Type column and the Results column or consider creating a subgroup of studies in Table 11 for those studies in which patients were suspected of having hair dye or hair care product allergy.

Summary – The EU regulations for these ingredients needs to be corrected. The Summary still states that the limit in hair dyes is 6% and that p-Phenylenediamine and its salts are only permitted in hair dyes for professional use. As stated in the cosmetic use section, 2% is the limit in hair dyes and there is no professional use limit for use in hair dyes.

Discussion – The Discussion currently states: "the Panel noted the lack of toxicity in acute and repeated dose toxicity studies". This is not correct. Toxicity is dose-dependent. There are many studies included in the CIR report that were completed using doses that caused adverse effects. The Discussion should note that there was a 90-day oral study that identified a NOAEL of 8 mg/kg/day and that this NOAEL is supported by other studies which found NOAEL or NOEL values of 5-10 mg/kg/day. The NOAEL of 8 mg/kg/day was used by the SCCS to calculate MoS values that were considered acceptable.

Please have the Expert Panel confirm the following sentence that is included in the Discussion. "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." Is there a reference that supports this statement?

Additional Considerations

Introduction – In the Introduction, it would be helpful to state the maximum concentration of use for p-Phenylenediamine in hair dyes that was reported in 2007.

Cosmetic Use – Since there are three ingredients included in this report, "This ingredient is considered a coal tar hair dye" needs to be corrected to "These ingredients are considered coal tar hair dyes".

Cosmetic Use – In the description of the SCCS opinion, it would be helpful to state why they say p-Phenylenediamine "remains a considerable concern for consumer safety" (because it is a potent contact allergen).

Non-Cosmetic Use, old report summary – The non-cosmetic uses reported in the original CIR report should be confirmed before including them in the amended CIR report. Searching for alfalfa meal and p-Phenylenediamine results in a lot of evidence that N,N-diphenyl-p-phenylenediamine was used in alfalfa meal but no evidence that p-Phenylenediamine was used. As the identified references concerning alfalfa meal are from the 1950's this use does not appear to be current. As "derivatives of p-Phenylenediamine" are not in this report, the last sentence of this section should be deleted.

Toxicokinetics, old report summary – As this section includes no information about doses or concentrations, it is not very useful. At a minimum, it would be helpful to include some numbers from the study that compared intravenous to dermal administration of p-Phenylenediamine in

rabbits, and some values of urinary recovery of radioactivity from persons using p-Phenylenediamine containing hair dyes.

Dermal Penetration, In Vitro – What was used as the receptor fluid?

ADME, In Vitro – The summary of reference 19 states: "The capacity for N-acetylation of p-Phenylenediamine in human skin samples was investigated." But the results just say that metabolism was observed. If they really looked at "capacity" it would be helpful to give some quantitative results. Was metabolism by the skin saturated at a specific dose/concentration of p-Phenylenediamine? If it was not a quantitative study, maybe "capacity" should be changed to "ability".

ADME, Animal, Othe Exposure Routes – This heading needs to be corrected to "Othe[r] Exposure Routes"

ADME, Human Dermal – In the last sentence of this section, it would be helpful to also indicate (if correct) that the C_{max}, T_{max} and AUC values were for radioactivity.

Acute Toxicity – Based on the information in Table 4, the minimal lethal dose in rats (reference 5, 7) was 75 mg/kg. The text says: "The minimal non-lethal oral dose of p-Phenylenediamine was 75 mg/kg in rats…" "non-lethal" needs to be changed to "lethal" or the dose needs to be changed to 50 mg/kg (which was the minimal non-lethal dose). It would be helpful if the text also noted that this was based on only 2 rats at 75 mg/kg and only 1 rat at 50 mg/kg.

Repeated Dose – Please state that the enzyme activities in the dermal guinea pig study (reference 5) were measured in the skin. For the 14-day oral study in rats (reference 9) please indicate the media in which lactate dehydrogenase and CPK were measured. This should also be clarified in Table 5.

Developmental and Reproductive Toxicity – Was a NOEL or NOAEL identified in the 90-day dermal study male rats?

Genotoxicity; Table 7 – Because hepatocytes have enzymes that metabolize xenobiotics, saying that a study using rat hepatocytes was done "without metabolic activation" is not correct. Hepatocytes are used for this type of study because they already have the potential to metabolize xenobiotics. This needs to be corrected in both the text and Table 7 (reference 5) ("without metabolic activation" should be deleted).

Carcinogenicity, old report summary – Please revise this summary so that all the information about the NCI dietary bioassay of p-Phenylenediamine is discussed together.

Carcinogenicity; Summary; Table 8 – What was the "test material" used in reference 47? "Glutathione 5-transferase" should be "glutathione S-transferase" as stated in the abstract of reference 46 (both the text and Table 8 need to be corrected). The text should also state that this is placental glutathione S-transferase (as stated in Table 8).

Hepatoxicity, old report summary – How was the lack of hepatic toxicity determined in the male rats given an intraperitoneal dose of p-Phenylenediamine?

Dermal Irritation and Sensitization; Summary; Table 9 – Table 9 indicates that ECHA did not consider the LLNA in guinea pigs to be a reliable study. As it is a non-standard study, perhaps it should be removed from the CIR report. There is sufficient evidence that p-Phenylenediamine is a strong sensitizer without this study. If it is left in the CIR report, the text should also note that ECHA did not consider it to be a reliable study.

Case Reports Related to Temporary Tattooing – Does FDA still have a "hotline" for reporting adverse reactions to temporary tattoos? Based on FDA's website, https://www.fda.gov/cosmetics/cosmetic-products/tattoos-temporary-tattoos-permanent-makeup adverse reactions should now be reported to FDA's Medwatch system. Perhaps this information should be added to this CIR report, or the information about the "hotline" removed from the report.

Clinical Reports of Cross-Sensitization – In the second last paragraph (reference 148), it would be helpful to state the substance which resulted in positive reactions in 2 control subjects.

Summary – In the Summary, it would be helpful to note that use of p-Phenylenediamine in eye makeup preparations is not permitted in the United States.

Summary – The Summary states: "The minimal and maximal non-lethal oral doses of p-Phenylenediamine were 75 mg/kg and 50 mg/kg, respectively, in rats…" This does not make sense. It should state: "The minimal [lethal] and maximal non-lethal oral doses of p-Phenylenediamine were 75 mg/kg and 50 mg/kg, respectively, in rats." As this was based on only 2 rats at 75 mg/kg and 1 rat at 50 mg/kg, it might be better to state that "one of two rats treated at 75 mg/kg died while the single rat treated at 50 mg/kg survived" rather than designating them as "minimum lethal" and "maximum non-lethal" doses.

Please add "In an inhalation study" before "the calculated LC_{50} for p-Phenylenediamine in rats was 0.92 mg/l…."

What was the concentration of p-Phenylenediamine and what species was used in the 4-month dermal study of a hair dye containing p-Phenylenediamine?

Please correct (add the word in brackets): "in a mitotic recombination [assay] when tested…"; "a dose-related [increase] in chromosomal aberrations"

Please add the frequency of treatment for the dermal DART studies.

It would be helpful to include some values for the rate of sensitization of hairdressers.

Discussion – Rather than saying "such use is not permitted", it would be helpful to be more specific and say "use in eye makeup products is not permitted".

Since the p-Phenylenediamine ingredients are not direct hair dyes, is the following sentence necessary in this report? "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."

Table 2 – Please add a molecular (or formula) weight for p-Phenylenediamine Sulfate.

Table 3 – For these hair dye ingredients with uses reported in a very limited number of product categories, the presentation of use information by "likely duration and exposure" is not helpful. Only the information by product category is necessary.

Table 4 – In the second dermal study, the test article column says: "p-Phenylenediamine applied as 40% aq. solution". Therefore, the Vehicle column should state "water" rather than "none".

In the second oral study, because only 1 or 2 rats were used in some of the treatment groups, it would be helpful to also include the numbers in the Results column, e.g., rather than saying "1 rat in the 100 mg/kg group died" it would be helpful to state that "the only rat treated with 100 mg/kg died".

Table 5 – In the third dermal study, what is meant by "blood examinations"? Did they measure hematological values, clinical chemistry, or both?

In the Protocol column of the 12-week oral study in rats it says that the main organs were examined histologically but the Results column says nothing about histological effects. What were the results of the histopathologic examinations especially the livers and kidneys, for which organ weights were increased?

Table 6 – In the first oral study, were the described effects observed at both doses? Please correct: "which resulted to degeneration" ("to" needs to be changed to "in")

Table 7 – When only one strain is positive in an Ames assay, it would be helpful if the results column noted that the other strains tested were negative. This needs to be revised for the following references 33, 30, 35, 38 (38 should state which strain(s) was positive).

Table 8 – The "subdermal" study should be moved to the "parenteral" section of the table with the subcutaneous study.

The abstract of reference 34 indicated that p-Phenylenediamine was studied after oxidation by hydrogen peroxide. This is not clear in Table 8. Because the subcutaneous portion of reference 34 is presented under "parenteral" it does not need to be under "dermal" (perhaps under dermal it should state that the subcutaneous portion of this study is presented under "parenteral").

Table 8, reference 26 – It is likely that body weight gains (rather than body weights) were less in treated rats. Please delete "in" in the following: "differences in among the groups"

Table 8, reference 5,7 – Please correct: "no adverse effects o[n] body weights" (add "n")

Table 12 – In the Presentation column it states: “popular eruptions” (reference 96) and “popular rash” (reference 105); “popular” needs to be changed to “papular” (this may be an autocorrect problem).

Reference 104 – Please add “s” to “calp”

Reference 105 – In the following, please change “incidence” to “incident”: “patient had dyed hair for 20 yr prior without incidence”

Table 13 – Since the first two studies in this table appear to be dermal use of black hair dye, it would be helpful to change the title of this table to “Case reports related to dermal hair dye and tattoo use”

Table 14 – After the two hairdresser ADME/exposure studies (references 150 and 153) are moved to the ADME section the rest of the studies concern effects, e.g., contact dermatitis, renal effects, in persons occupationally exposed to p-Phenylenediamine. The title of this table needs to be changed to “Assessment of Effects in Persons Occupationally Exposed to p-Phenylenediamine”.

Reference 155 – It should also be noted that adverse kidney effects have also been associated with exposure to henna.

***p*-Phenylenediamine - June 2024 – Christina Burnett**	
Comment Submitter: Alexandra Kowcz, Personal Care Products Council **Date of Submission: January 23, 2024**	
Comment	**Response/Action**
Key Issue: ADME, Human Dermal; Table 14 – There are two studies in Table 14 regarding exposure and ADME in hairdressers. These studies (references 150 and 153) should be presented in the ADME section. The study in 128 hairdressers (reference 150) is a study that provides measures of exposure used to estimate daily exposure (<0.36 µg p-Phenylenediamine$_{eq}$/kg/bw/work day) in hairdressers that completed 6 hair dying processes in a day.	Moved studies per Council comment.
Key Issue: Ocular Irritation; Summary; Table 10 – The ECHA dossier rated the 3-month ocular test as not reliable. Please consider removing this study from the CIR report. In addition, the ECHA dossier says that the test substance was a hair dye containing p-Phenylenediamine. The dossier does not say the concentration of p-Phenylenediamine in the hair dye. The dossier says that 5, 10, or 15% of the test substance was instilled into the eyes of rats daily for up to 3 months, in contrast to the CIR report which incorrectly states that "rats received up to 15% p-Phenylenediamine in formulation" – the concentration of p-Phenylenediamine is not stated in the dossier. The concentration(s) of hair dye resulting in keratitis and corneal opacities was not clearly stated in the ECHA dossier, while the text of the CIR report suggests that it was at 15%. If this study is left in the CIR report, the description should be corrected so that it is consistent with the information in the ECHA dossier.	Corrections made describing concentrations of material tested. It is up to the Panel on whether this study remains in the safety assessment.
Key Issue: Multicenter and Retrospective Studies; Summary; Table 11 – Please review the studies in Table 11 again. Studies with the very high rates of sensitization, such as 67.5% in patients from India, were studies of patients with suspected hair dye allergy, or suspected hair care product allergy. It would be helpful if this was stated in the text. In Table 11, it would be helpful to identify the patient selection criteria in both the Clinical Testing Type column and the Results column or consider creating a subgroup of studies in Table 11 for those studies in which patients were suspected of having hair dye or hair care product allergy	Additional information added to the body of the report and the table now includes an extra column denoting if it is known if the patients had known to have suspected hair dye allergy.
Key Issue: Summary – The EU regulations for these ingredients needs to be corrected. The Summary still states that the limit in hair dyes is 6% and that p-Phenylenediamine and its salts are only permitted in hair dyes for professional use. As stated in the cosmetic use section, 2% is the limit in hair dyes and there is no professional use limit for use in hair dyes.	Correction made to Summary.
Key Issue: Discussion – The Discussion currently states: "the Panel noted the lack of toxicity in acute and repeated dose toxicity studies". This is not correct. Toxicity is dose-dependent. There are many studies included in the CIR report that were completed using doses that caused adverse effects. The Discussion should note that there was a 90-day oral study that identified a NOAEL of 8 mg/kg/day and that this NOAEL is supported by other studies which found NOAEL or NOEL values of 5-10 mg/kg/day. The NOAEL of 8 mg/kg/day was used by the SCCS to calculate MoS values that were considered acceptable. Please have the Expert Panel confirm the following sentence that is included in the Discussion. "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." Is there a reference that supports this statement?	Discussion updated with wording on dose-dependent toxicity and noting the NOAEL. Panel asked for discussion items from the 2007 report to be carried over into this current safety assessment. Panel will need to determined if this statement is still valid and if there needs to be further development in the body of the report.
Introduction – In the Introduction, it would be helpful to state the maximum concentration of use for p-Phenylenediamine in hair dyes that was reported in 2007.	Edit not accepted. Not standard procedure in Introduction and the information may be found in the Use section and summary.

***p*-Phenylenediamine - June 2024 – Christina Burnett**	
Comment Submitter: Alexandra Kowcz, Personal Care Products Council **Date of Submission: January 23, 2024**	
Comment	**Response/Action**
Cosmetic Use – Since there are three ingredients included in this report, "This ingredient is considered a coal tar hair dye" needs to be corrected to "These ingredients are considered coal tar hair dyes".	Edits accepted.
Cosmetic Use – In the description of the SCCS opinion, it would be helpful to state why they say p-Phenylenediamine "remains a considerable concern for consumer safety" (because it is a potent contact allergen).	Edit accepted.
Non-Cosmetic Use, old report summary – The non-cosmetic uses reported in the original CIR report should be confirmed before including them in the amended CIR report. Searching for alfalfa meal and p-Phenylenediamine results in a lot of evidence that N,N-diphenyl-p-phenylenediamine was used in alfalfa meal but no evidence that p-Phenylenediamine was used. As the identified references concerning alfalfa meal are from the 1950's this use does not appear to be current. As "derivatives of p-Phenylenediamine" are not in this report, the last sentence of this section should be deleted.	Sentence deleted as suggested.
Toxicokinetics, old report summary – As this section includes no information about doses or concentrations, it is not very useful. At a minimum, it would be helpful to include some numbers from the study that compared intravenous to dermal administration of p-Phenylenediamine in rabbits, and some values of urinary recovery of radioactivity from persons using p-Phenylenediamine containing hair dyes.	Additional detail added.
Dermal Penetration, In Vitro – What was used as the receptor fluid?	Normal saline solution. Added to paragraph.
ADME, In Vitro – The summary of reference 19 states: "The capacity for N-acetylation of p-Phenylenediamine in human skin samples was investigated." But the results just say that metabolism was observed. If they really looked at "capacity" it would be helpful to give some quantitative results. Was metabolism by the skin saturated at a specific dose/concentration of p-Phenylenediamine? If it was not a quantitative study, maybe "capacity" should be changed to "ability".	Additional details provided.
ADME, Animal, Othe Exposure Routes – This heading needs to be corrected to "Othe[r] Exposure Routes"	Correction made.
ADME, Human Dermal – In the last sentence of this section, it would be helpful to also indicate (if correct) that the C_{max}, T_{max} and AUC values were for radioactivity.	Edit made.
Acute Toxicity – Based on the information in Table 4, the minimal lethal dose in rats (reference 5, 7) was 75 mg/kg. The text says: "The minimal non-lethal oral dose of p-Phenylenediamine was 75 mg/kg in rats…" "non-lethal" needs to be changed to "lethal" or the dose needs to be changed to 50 mg/kg (which was the minimal non-lethal dose). It would be helpful if the text also noted that this was based on only 2 rats at 75 mg/kg and only 1 rat at 50 mg/kg.	Correction made, deleted "non-". Added the n for the 75 mg/kg dose.
Repeated Dose – Please state that the enzyme activities in the dermal guinea pig study (reference 5) were measured in the skin. For the 14-day oral study in rats (reference 9) please indicate the media in which lactate dehydrogenase and CPK were measured. This should also be clarified in Table 5.	Edits made.
Developmental and Reproductive Toxicity – Was a NOEL or NOAEL identified in the 90-day dermal study male rats?	Added "NOEL/NOAEL not identified".
Genotoxicity; Table 7 – Because hepatocytes have enzymes that metabolize xenobiotics, saying that a study using rat hepatocytes was done "without metabolic activation" is not correct. Hepatocytes are used for this type of study because they already have the potential to metabolize xenobiotics. This needs to be corrected in both the text and Table 7 (reference 5) ("without metabolic activation" should be deleted).	Corrections made.

***p*-Phenylenediamine - June 2024 – Christina Burnett**	
Comment Submitter: Alexandra Kowcz, Personal Care Products Council **Date of Submission: January 23, 2024**	
Comment	**Response/Action**
Carcinogenicity, old report summary – Please revise this summary so that all the information about the NCI dietary bioassay of p-Phenylenediamine is discussed together.	Edits made.
Carcinogenicity; Summary; Table 8 – What was the "test material" used in reference 47? "Glutathione 5-transferase" should be "glutathione S-transferase" as stated in the abstract of reference 46 (both the text and Table 8 need to be corrected). The text should also state that this is placental glutathione S-transferase (as stated in Table 8).	Corrections made.
Hepatoxicity, old report summary – How was the lack of hepatic toxicity determined in the male rats given an intraperitoneal dose of p-Phenylenediamine?	No further details provided in original report.
Dermal Irritation and Sensitization; Summary; Table 9 – Table 9 indicates that ECHA did not consider the LLNA in guinea pigs to be a reliable study. As it is a non-standard study, perhaps it should be removed from the CIR report. There is sufficient evidence that p-Phenylenediamine is a strong sensitizer without this study. If it is left in the CIR report, the text should also note that ECHA did not consider it to be a reliable study.	Added text to describe why the study was not reliable.
Case Reports Related to Temporary Tattooing – Does FDA still have a "hotline" for reporting adverse reactions to temporary tattoos? Based on FDA's website, https://www.fda.gov/cosmetics/cosmetic-products/tattoos-temporary-tattoos-permanent-makeup adverse reactions should now be reported to FDA's Medwatch system. Perhaps this information should be added to this CIR report, or the information about the "hotline" removed from the report.	Hotline is still listed. Direct by Bart Heldreth not to change.
Clinical Reports of Cross-Sensitization – In the second last paragraph (reference 148), it would be helpful to state the substance which resulted in positive reactions in 2 control subjects.	Edits accepted.
Summary – In the Summary, it would be helpful to note that use of p-Phenylenediamine in eye makeup preparations is not permitted in the United States.	Edits accepted.
Summary – The Summary states: "The minimal and maximal non-lethal oral doses of p-Phenylenediamine were 75 mg/kg and 50 mg/kg, respectively, in rats…" This does not make sense. It should state: "The minimal [lethal] and maximal non-lethal oral doses of p-Phenylenediamine were 75 mg/kg and 50 mg/kg, respectively, in rats." As this was based on only 2 rats at 75 mg/kg and 1 rat at 50 mg/kg, it might be better to state that "one of two rats treated at 75 mg/kg died while the single rat treated at 50 mg/kg survived" rather than designating them as "minimum lethal" and "maximum non-lethal" doses. Please add "In an inhalation study" before "the calculated LC50 for p-Phenylenediamine in rats was 0.92 mg/l…." What was the concentration of p-Phenylenediamine and what species was used in the 4-month dermal study of a hair dye containing p-Phenylenediamine? Please correct (add the word in brackets): "in a mitotic recombination [assay] when tested…"; "a dose-related [increase] in chromosomal aberrations". Please add the frequency of treatment for the dermal DART studies. It would be helpful to include some values for the rate of sensitization of hairdressers.	Edits accepted.

***p*-Phenylenediamine - June 2024 – Christina Burnett**	
Comment Submitter: Alexandra Kowcz, Personal Care Products Council **Date of Submission: January 23, 2024**	
Comment	**Response/Action**
Discussion – Rather than saying "such use is not permitted", it would be helpful to be more specific and say "use in eye makeup products is not permitted". Since the p-Phenylenediamine ingredients are not direct hair dyes, is the following sentence necessary in this report? "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."	Edit addressed. Boilerplate language, but an additional sentence has been added to clarify the type of dye p-Phenylenediamine is.
Table 2 – Please add a molecular (or formula) weight for p-Phenylenediamine Sulfate.	Added.
Table 3 – For these hair dye ingredients with uses reported in a very limited number of product categories, the presentation of use information by "likely duration and exposure" is not helpful. Only the information by product category is necessary.	Panel has stated this is their preferred format for the Use Table. Any edits to this format must come at Panel direction.
Table 4 – In the second dermal study, the test article column says: "p-Phenylenediamine applied as 40% aq. solution". Therefore, the Vehicle column should state "water" rather than "none". In the second oral study, because only 1 or 2 rats were used in some of the treatment groups, it would be helpful to also include the numbers in the Results column, e.g., rather than saying "1 rat in the 100 mg/kg group died" it would be helpful to state that "the only rat treated with 100 mg/kg died".	All edits accepted.
Table 5 – In the third dermal study, what is meant by "blood examinations"? Did they measure hematological values, clinical chemistry, or both? In the Protocol column of the 12-week oral study in rats it says that the main organs were examined histologically but the Results column says nothing about histological effects. What were the results of the histopathologic examinations especially the livers and kidneys, for which organ weights were increased?	No further details were provided. Details added on histopathological findings.
Table 6 – In the first oral study, were the described effects observed at both doses? Please correct: "which resulted to degeneration" ("to" needs to be changed to "in")	Edits accepted.
Table 7 – When only one strain is positive in an Ames assay, it would be helpful if the results column noted that the other strains tested were negative. This needs to be revised for the following references 33, 30, 35, 38 (38 should state which strain(s) was positive).	Edits made; no further details available in reference 38.
Table 8 – The "subdermal" study should be moved to the "parenteral" section of the table with the subcutaneous study. The abstract of reference 34 indicated that p-Phenylenediamine was studied after oxidation by hydrogen peroxide. This is not clear in Table 8. Because the subcutaneous portion of reference 34 is presented under "parenteral" it does not need to be under "dermal" (perhaps under dermal it should state that the subcutaneous portion of this study is presented under "parenteral").	All edits accepted.
Table 8, reference 26 – It is likely that body weight gains (rather than body weights) were less in treated rats. Please delete "in" in the following: "differences in among the groups"	Edits accepted.
Table 8, reference 5,7 – Please correct: "no adverse effects o[n] body weights" (add "n")	Corrected.

***p*-Phenylenediamine - June 2024 – Christina Burnett**	
Comment Submitter: Alexandra Kowcz, Personal Care Products Council **Date of Submission: January 23, 2024**	
Comment	**Response/Action**
Table 12 – In the Presentation column it states: "popular eruptions" (reference 96) and "popular rash" (reference 105); "popular" needs to be changed to "papular" (this may be an autocorrect problem). Reference 104 – Please add "s" to "calp" Reference 105 – In the following, please change "incidence" to "incident": "patient had dyed hair for 20 yr prior without incidence"	All edits accepted.
Table 13 – Since the first two studies in this table appear to be dermal use of black hair dye, it would be helpful to change the title of this table to "Case reports related to dermal hair dye and tattoo use"	Title changed
Table 14 – After the two hairdresser ADME/exposure studies (references 150 and 153) are moved to the ADME section the rest of the studies concern effects, e.g., contact dermatitis, renal effects, in persons occupationally exposed to p-Phenylenediamine. The title of this table needs to be changed to "Assessment of Effects in Persons Occupationally Exposed to p-Phenylenediamine". Reference 155 – It should also be noted that adverse kidney effects have also been associated with exposure to henna.	Title changed, edits accepted.

WVE
WOMEN'S VOICES FOR THE EARTH

April 26, 2024

RE: Comments on the Safety Assessment of PPD

To the CIR:

I am writing on behalf of Women's Voices for the Earth to provide the following comments on the safety assessment of PPD as Used in Cosmetics. Women's Voices for the Earth works to reduce the use of toxic chemicals in professional and retail beauty care products. We are concerned that the current draft safety assessment is not protective of public health for the following reasons:

- The maximum concentration of use listed in the safety assessment is too low. Additional government data on PPD levels in hair dyes is available indicating a maximum concentration of 29.9% (instead of 3% as listed in the draft safety assessment).
- An ingredient known to cause sensitization (a lifelong condition) from intended conditions of use should not be considered "safe for use".
- Caution statements and patch test instructions are ineffective at establishing safe use.
    - Research demonstrates that patch tests are rarely conducted before hair dye use.
    - Even when patch tests are conducted, they are not effective in preventing adverse reactions.
- Studies on severe health effects caused by PPD exposure from hair dye are missing from the safety assessment. (Specifically, they are absent from Table 12: Case reports related to hair dye use.)
    - Documented case reports of health effects from PPD exposure from hair dye use include chronic renal failure, acute hepatitis, renal impairment, contact anaphylaxis, severe immediate hypersensitivity, hypertrophic allergic contact dermatitis.

1) The maximum concentration of use listed in the safety assessment is too low. Additional government data on PPD levels in hair dyes available in the U.S. should be included in the safety assessment.

The range of concentration of use of PPD in the current draft of the safety assessment is currently incomplete. Additional data on actual levels of PPD in cosmetic products is available from the EU SafetyGate database[1]. The European Union maintains the SafetyGate database – which reports on governmental chemical testing of products (including cosmetics) where chemical levels are detected at higher than allowed amounts. As the EU has a maximum allowed level of PPD of 6%, there are over 20 cosmetic products (largely henna-based hair dyes) that have been reported to the database in recent years (and as a result banned from sale in the EU) **which contain considerably higher levels of PPD – up**

[1] https://ec.europa.eu/safety-gate/#/screen/home?lang=en

to 29.9%. The data for products recently detected is available in the Table 1 (at the end of these comments), with links to the Safety Gate report with photos of each reported product. Many of these products detected in the EU are also available in the United States either in stores which have imported the product – or for sale on Amazon.com. Here is just one example:

Black Rose Kali Mehandi hair dye (12.64% PPD):

EU SafetyGate Database report (with photo of product packaging) indicating the product contains 12.64% PPD

https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/10007687

The same product (Black Rose Kali Mehandi hair dye) – sold on Amazon.com to US customers for $9.62

https://www.amazon.com/Sachets-Black-Mehandi-Henna-Herbal/dp/B077ZQXNF7

See Table 1 at the end of this document for the full list of products and their PPD concentrations.

Consequently, the Margins of Safety (MOS) in the safety assessment should also be recalculated to use a more conservative concentration of use. The current calculations use a 2% concentration, when the actual concentration from some products could be 10-15 times that level.

2) An ingredient known to cause sensitization (a lifelong condition) from intended conditions of use should not be considered "safe for use".

The 1985 CIR Safety Assessment was very clear in stating that

> *"p-Phenylenediamine is a known sensitizer and that some persons may be sensitized under intended conditions of use."*

There is no new data presented in the safety assessment that contradicts this statement. It seems clear that using cosmetics containing PPD as intended will cause some users to develop sensitization to PPD. It is also clear that cosmetic use – especially hair dye use – is the main cause of the high rates of sensitization to PPD demonstrated in the dermatological literature. There are many thousands of cosmetic users in the US currently sensitized to PPD, caused by their prior use of hair dye. Even the rates of PPD sensitization in young children has increased. A recent paper on allergic contact dermatitis due to PPD found:

> *"Among the allergens involved in ACD, the frequency of paraphenylenediamine (PPDA) is increasing. PPDA is one of the five most common contact allergens in the general population and one of the 10 most common contact allergens in children. The most relevant sources today are henna tattoos and hair dyes."*[2].

[2] Alberola IA, Hernández EJ, Carracedo JFS, Panedas AM, Iranzo NM, Morate MB, López SA, Calleja JR. Early presentation of allergic contact dermatitis due to paraphenylenediamine. Allergol Immunopathol (Madr). 2024 Jan 1;52(1):93-96. doi: 10.15586/aei.v52i1.89 https://pubmed.ncbi.nlm.nih.gov/38186199/

Hairdressers and cosmetologists also have drastically higher rates of sensitization than people in other occupations. There are many more people not yet sensitized to PPD, but who are destined to become sensitized to PPD simply from using hair dyes as instructed by the manufacturers. Thousands of future hair dye users, responsibly adhering to all hair dye instructions, will unnecessarily suffer this lifelong dermatological condition because manufacturers have been told by the CIR that PPD is a safer ingredient to include in hair dyes.

CIR expert panel members should not conclude a cosmetic ingredient to be "safe as used" when it is known to cause sensitization in users (including children) when used as intended.

3.) Caution statements and patch test instructions are ineffective at establishing safe use.

The CIR appears to be relying on the fact that hair dyes are required to include a caution statement and patch test instructions to help determine if a product would cause skin irritation. The CIR safety assessment Discussion section states:

> *"The Panel expects that following this procedure (i.e. patch tests) will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures."*

Unfortunately, the caution statements and patch test instructions have been clearly demonstrated to be ineffective in avoiding skin reactions to hair dyes.

A 2022 study of 355 hair dye users reported just 18.9% of users who had ever completed an allergy test before hair dye use[3]. This study further assessed whether those who had ever completed an allergy test were able to avoid skin reactions, and there was a non-significant difference in the results:

- 17% of those who did an allergy pre-test still reported side effects of hair dye.
- 20% of those who did not do an allergy pre-test reported side effects of hair dye.

In other words, this study showed that even those few who did conduct an allergy test were just as likely to report side-effects of the hair dye – rendering the allergy patch test ineffective as a safety procedure.

Secondly – in practice, we know that hair dye users rarely pay attention to caution statements and rarely conduct patch tests prior to use of hair dye. This has been repeatedly demonstrated in the

[3] Al Dhafiri, M., Al Furaikh, B., Aljasir, A., Al Dandan, A., Alfalah, R.m, Albahar, S. Practice and Impact of Hair Dyeing; A Local Study. International Journal of Innovative Research in Medical Science. Vol. 7, No. 12 (2022). doi:10.23958/ijirms/vol07-i12/1594 https://ijirms.in/index.php/ijirms/article/view/1594

literature. In a study of 271 PPD-sensitive patients, 80% had used hair dye, but of those only **3.6**% had ever conducted a pre-test.[4]

In a survey of 263 volunteers who used hair dye, it stated:

*"90% of the volunteers never performed a skin test prior to the use compared to **10%** who did skin test at times."*[5]

The CIR Expert Panel members cannot ignore the reality of most hair dye user behaviors in determining the safety of a cosmetic ingredient.

4) Studies on severe health effects caused by PPD exposure from hair dye are missing from the safety assessment. (Specifically, they are absent from Table 12: Case reports related to hair dye use.)

There are many case studies reported in the literature demonstrating severe health effects from exposure to PPD from hair dye use. Health effects reported include chronic renal failure, acute hepatitis, contact anaphylaxis, severe immediate hypersensitivity and permanent leukoderma. None of these case studies (or these health effects) are mentioned in the current draft safety assessment. (The safety assessment does mention that there are case reports of PPD intoxication from accidental or intentional ingestion and dismisses them as irrelevant. But here I present relevant case reports of severe health effects from the use of hair dye containing PPD that should be included in the safety assessment.)

Case studies of exposure to PPD in hair dye which should be added to Table 12.

Brown JH, McGeown MG, Conway B, Hill CM. **Chronic renal failure associated with topical application of paraphenylenediamine.** Br Med J (Clin Res Ed). 1987 Jan 17;294(6565):155. doi: 10.1136/bmj.294.6565.155.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1245164/?page=1

"Paraphenylenediamene ("para"), a derivative of paranitroaniline, is widely used as a hair dye…We report two cases of vasculitis and crescentic glomerulonephritis after prolonged topical application."

(Note: the citation to this case study report is already included in the References section (#99) but the findings of the study are never mentioned in the text and is not included in Table 12.)

[4] Schubert S, Lessmann H, Schnuch A, Uter W, Geier J; IVDK. Factors associated with p-phenylenediamine sensitization: data from the Information Network of Departments of Dermatology, 2008-2013. Contact Dermatitis. 2018 Mar;78(3):199-207. doi: 10.1111/cod.12920. https://pubmed.ncbi.nlm.nih.gov/29322532/

[5] Patel D, Narayana S, Krishnaswamy B. Trends in use of hair dye: a cross-sectional study. Int J Trichology. 2013 Jul;5(3):140-3. doi: 10.4103/09747753.125610. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3927172/

Alice Tseng, Colin Kovacs and David K.H. Wong. When beauty is more than skin deep: **Acute hepatitis secondary to topical para-phenylenediamine exposure from hair dye shampoo** Canadian Family Physician June 2023, 69 (6) 403-405; https://doi.org/10.46747/cfp.6906403 https://www.cfp.ca/content/69/6/403.long

"We describe the first reported case of reversible acute hepatitis related to PPD-containing hair dye shampoo."

Hamdouk M, Abdelraheem M, Taha A, Cristina D, Checherita IA, Alexandru C. **The association between prolonged occupational exposure to paraphenylenediamine (hair-dye) and renal impairment**. Arab J Nephrol Transplant. 2011 Jan;4(1):21-5. doi: 10.4314/ajnt.v4i1.63151.

https://pubmed.ncbi.nlm.nih.gov/21469591/

"Conclusion: In this group of hairdressers with regular exposure to PPD, we observed high prevalence of renal impairment, proteinuria and hematuria. These findings were significantly associated with the use of pure forms of PPD and longer duration of exposure."

Bai YH, Peng YM, Yin WQ, Liu H, Liu FY, Duan SB, Xiao P. p-Aminophenol and **p-paraphenylenediamine induce injury and apoptosis of human HK-2 proximal tubular epithelial cells**. J Nephrol. 2012 Jul-Aug;25(4):481-9. doi: 10.5301/JN.2011.8495.

https://pubmed.ncbi.nlm.nih.gov/21786225/

(Note: This study is not a case report, but, relevantly, researchers for this paper state: *"There is rapidly accumulating evidence that use of oxidized hair dye causes various forms of nephrotoxic injury."* Their research provides evidence of a mechanism of action for this health effect from PPD exposure.)

Fukunaga T, Kawagoe R, Hozumi H, Kanzaki T. **Contact anaphylaxis due to para-phenylenediamine**. Contact Dermatitis. 1996 Sep;35(3):185-6. doi: 10.1111/j.1600-0536.1996.tb02348.

https://pubmed.ncbi.nlm.nih.gov/8930490/

Nosbaum A, Dupin C, Nicolas JF, Bérard F. **Severe immediate hypersensitivity and allergic contact dermatitis caused by hair dyes**. Contact Dermatitis. 2012 Jul;67(1):52-3. doi: 10.1111/j.1600-0536.2012.02076.x.

https://pubmed.ncbi.nlm.nih.gov/22681466/

Wong GA, King CM. **Immediate-type hypersensitivity and allergic contact dermatitis due to para-phenylenediamine in hair dye**. Contact Dermatitis. 2003 Mar;48(3):166. doi: 10.1034/j.1600-0536.2003.00038.x.

https://pubmed.ncbi.nlm.nih.gov/12755736/

Santucci B, Cristaudo A, Cannistraci C, Amantea A, Picardo M. **Hypertrophic allergic contact dermatitis from hair dye**. Contact Dermatitis. 1994 Sep;31(3):169-71. doi: 10.1111/j.1600-0536.1994.tb01958.x.

https://pubmed.ncbi.nlm.nih.gov/7821010/

While case reports are anecdotal in nature, it is unclear what the actual prevalence of these events may be. (How many people have suffered from acute hepatitis, renal failure or hypersensitivity without their medical providers connecting it to their recent use of hair dye and further documenting the connection?) **The CIR must decide if it is worth allowing these serious health events to happen to even a few people just for the sake of dying hair with a particular ingredient.** In any case, the CIR safety assessment must include these case reports so that readers better understand what potential health risks are considered "acceptable" in a CIR determination of "safe as used".

Thank you for your consideration of these comments.

Sincerely,

Alexandra Scranton
Director of Science and Research
Women's Voices for the Earth

Table 1: EU Reports of Para-phenylenediamene detected in hair dye, brow dye and henna products 2005-2023, Serious risk posed by high levels

Source: Safety Gate : the EU rapid alert system for dangerous non-food products

https://ec.europa.eu/safety-gate/#/screen/home?lang=en

Alert number	PPD % detected	Product/ Name	Description	Brand	URL of Case
1732/10	29.9%	Natural henna set - Dogul Kina Seti	Green powder, black powder	Gökçehan	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/-17998
0610/05	26.0%	Name: Black powder HENNA TATTOO			https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/-1677
0006/10	15.3%	Herbal henna - Moon Star Herbal Henna black	Herbal henna.	Moon Star	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/-16296
0780/07	13.7%	Permanent powder hair colour	White-gold cartoon box containing a bottle with powder	BIGEN	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/-12483
0402/06	13.1%	Permanent Powder Hair Dye JET BLACK "Sta Sof Fro"		Sta Sof Fro	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/-11227
A12/00239/23	12.8%	Hair dye - Black Rose	Herbal based black henna in the form of powder.	Black Rose	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/10007688

A12/00238/23	12.6%	Hair dye - Black Rose	Black powder hair dye.	Kali Mehandi	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/10007687
A12/0147/13	12.3%	Hair dye	Herbal Henna	Moon Star	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/59575
1949/10	12.0%	Hair dye (Herbal Naturally Black Henna)	Olive-coloured powder.	Herbal	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/-18208
A12/0862/12	10.9%	Black Henna (hair dye)	aluminium foil sachet containing 10g of the product.	Afrin's	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/34589
A12/02557/23	10.2%	Hair dye	Henna-based hair dye.	Shringar Black Henna	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/10010578
A12/1601/18	10.0%	Hair dye. Permanent Powder Hair Colour	Grey powder to dye hair black	Bigen Hoyu 58 Oriental Black	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/332145
A12/1609/19	9.8%	Hair dye Premium Quality Henna Powder	30 g of hair colouring powder	MAYURI HENNA natural black	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/388748
A12/00938/22	9.7%	Hair dye	Balck Henna hair dye.	Royal	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/10006347
A12/1036/12	9.3%	Royal Black Henna	6 x 10g sachets of powder hair dye.	Royal	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/38827
A12/00629/22	8.8%	Eyebrow tint - Uncut Henna for Brows	Henna eyebrow dye (dark brown)	Uncut Henna. Dark Brown	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/10005881
A12/0148/13	8.7%	Hair dye	Herbal Henna	Moon Star Copper Brown	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/59589
A12/0604/18	7.9%	Hair colouring product - Kali	Henna hair colouring product.	Black Rose	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/309615

		Mehandi/ Black Henna			
A12/0009 3/20	7.6%	Hair dye - Black Henna (Henna hair colour)	60 g of hair dye powder	Royal	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/10000320
A12/0004 4/19	7.3%	Hair dye - HENNA HAIR COLOR	Powder for hair dyeing,	Henna Vital Black/Siyah Henna	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/10000011
A12/1832 /19	7.3%	Brow Henna medium brown	Brow colouring powder,	AS Lashes medium brown	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/395550
A12/0071 5/23	6.4%	Hair dye - Vatika Hair dye colour	Henna hair dyes, brown homogeneous powder	Vatika	https://ec.europa.eu/safety-gate-alerts/screen/webReport/alertDetail/10008378

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, M.S., Senior Scientific Analyst/Writer, CIR
Jinqiu Zhu, PhD, DABT, ERT, DCST, CIR Toxicologist
Date: May 10, 2024
Subject: Response to the WVE comments on *p*-Phenylenediamine

The enclosed comments received from Women's Voices for the Earth (WVE), dated April 26, 2024, on the Tentative Amended Report of *p*-Phenylenediamine ingredients are submitted for the Panel's review.

In their comments, WVE provided a list of hair dye products containing *p*-Phenylenediamine with concentrations ranging from 6.4% to 29.9%. These products were flagged in the EU Safety Gate alert system, with a note indicating their non-compliance with the Cosmetic Products Regulation at the EU level. Additionally in their comments, WVE argued that an ingredient known to cause sensitization—a lifelong condition—under its intended use conditions should not be deemed "safe for use." They also mentioned that caution statements and patch test instructions on hair dye product labels do not effectively ensure safe use. Finally, WVE requested the inclusion of several additional case studies in the report.

The Panel's review of the safety of cosmetic ingredients is grounded in *the present practices of use and concentrations as described in the safety assessment report*. Therefore, the Panel's conclusions on ingredients are also based on the concentration and frequency of use information that is presented in the report and comes from a validated source. According to the Council's 2022 survey, *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%.[1] The CIR staff has checked the list of hair dye products submitted by WVE and determined that these products are manufactured globally in countries such as India, Turkey, and South Africa, rather than in the US. For example, the product highlighted by WVE, the *Black Rose Kali Mehandi* hair dye available on Amazon is produced in Austria. However, the Amazon product page does not disclose the concentration of *p*-Phenylenediamine in the formulation. This product was tested by a regulatory agent in the EU and found to contain 12.64% *p*-Phenylenediamine (by weight), and consequently marked as "The product does not comply with the Cosmetic Products Regulation" in the EU Safety Gate alert system. (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.[2])

While the safety assessment on *p*-Phenylenediamine ingredients is still in Draft Final Amended Report status, the Panel's potential conclusion must always be based on the usage practices and concentrations documented in the report, i.e., for this report, at concentrations ranging from 0.98 to 3% *p*-Phenylenediamine in hair dyes, with a maximum on-head concentration of 1% after dilution. Accordingly, if a product is marketed with ingredient use conditions or concentrations exceeding those described in the Panel's safety assessment, their conclusion is not applicable to that product (i.e., a Panel conclusion of "safe in the present practices of use and concentrations as described in this safety assessment" is not at all a blanket statement of "safe for use.").

Regarding WVE's argument that an ingredient known to cause sensitization cannot be considered "safe for use," in addition to the explanation just provided that CIR conclusions are not a blanket statement of safe for use, it is generally understood that individuals sensitized to certain substances should avoid exposure to those substances but people not sensitized to those same substances can safely be exposed to them. For instance, while some people may be allergic to peanuts or penicillin, this does not mean that peanuts cannot be safely consumed by others or that penicillin cannot be used effectively and safely to treat infections in

patients who are not allergic. As stated in the Introduction of the report, 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 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."*[5] Therefore, "safe for use" should be understood as applicable to those who are not sensitized. Furthermore, it is well recognized that the concentration of an ingredient in products also plays an important role in skin sensitization. The No Expected Sensitization Induction Level (NESIL) is determined to specify the amount of an ingredient to which one can be exposed without causing skin sensitization.

A study on the occupational exposure of hairdressers to hair dyes that are associated with the development of allergic contact dermatitis (ACD) reported a NESIL of 27.5 μg/cm² for *p*-Phenylenediamine.[6] In this research, the Measured Exposure Level (MEL) of *p*-Phenylenediamine was quantified by summing the amounts of radio-labeled [^{14}C]-*p*-Phenylenediamine found in the stratum corneum, epidermis, dermis, and receptor fluid. The hair dye product used in the study contained 2% *p*-Phenylenediamine after being mixed with the developer, aligning with the maximum allowable use concentration in the EU. The dose of hair dye product applied to human skin was 445.6 ± 39.2 μg/cm² per application. The results further indicated that estimated hairdresser hand exposure level is 2.7-fold below the individual NESIL (at 27.5 μg/cm²) for *p*-Phenylenediamine. Furthermore, it should be noted in the SCCS's Opinion on *p*-Phenylenediamine,[7] the highest cumulative penetration obtained in relevant studies on percutaneous absorption of *p*-Phenylenediamine was 4.47 μg/cm² (based on cumulative mass absorbed per scalp at 3129 μg and an estimated scalp area of 700 cm²), leading to a margin of safety (MOS) of 200 in their calculation.

The US FD&C Act mandates the inclusion of caution statements on hair dye products and requires labeled directions to instruct consumers to conduct a skin test.[8] The US FDA has the authority to take action against a cosmetic on the market if it contains a poisonous or deleterious substance that could harm consumers when used in the customary or expected way and used according to labeled directions. As highlighted by the US FDA, *it's important to follow the directions on the label; it is also important to be an informed consumer and understand the risks.*[8] The Panel does not engage in regulatory affairs. The purview of the Panel is to assess the safety of the ingredients based on scientific evidence under intended use conditions, considering the current usage practices and concentrations as detailed in reports.

Finally, WVE submitted a list of case studies and requested their inclusion in the report. It deserves to be noted that the CIR 2007 Amended Report on *p*-Phenylenediamine included approximately **80** case studies.[9] From the list provided by WVE, three papers (Wong et al., 2003; Fukunaga et al., 1996; and Brown et al., 1987) were already covered in that report, and two of these have now been presented in this iteration of the Draft Final Amended Report (exception: Brown et al., 1987 study. In this study, a mixture of *p*-Phenylenediamine and henna was applied. As the authors clarified in the paper, *henna is used by the Islamic community to dye hair, skin, and nails for important occasions. The traditional method of application requires that the woman (or bridegroom) remains immobile for about* ***six hours*** *on two successive days, but this process can be reduced to a matter of minutes by adding paraphenylenediamine to the henna.* Therefore, in this study, *p*-Phenylenediamine was not applied under its intended use conditions as hair dye.) Additionally, it should be noted that the findings from Santucci et al., 1994 and Hamdouk et al., 2011 had already been summarized in Table 13 and Table 14, respectively. Furthermore, Bai et al., 2012 is not a case study but an in vitro cytotoxicity study – the results are now available in the appropriate section of the report. The results from the remaining two papers (Tseng et al., 2023 and Nosbaum et al., 2011) are also now incorporated into this Draft Final Amended Report in Table 12.

The Panel is requested to review WVE's comments and take them into consideration during their discussion of the report on *p*-Phenylenediamine.

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8. U.S. Food and Drug Administration (FDA). Hair Dyes. https://www.fda.gov/cosmetics/cosmetic-products/hair-dyes. Updated 02/25/2022. Accessed.
9. Johnson WJ, Bergfeld WF, Belsito DV, et al. *Amended Final Report of the Safety Assessment of p-Phenylenediamine, p-Phenylenediamine HCl, and p-Phenylenediamine Sulfate.* Washington, DC. 2007.

***p*-Phenylenediamine and Its Salts History**

1985– The CIR's Final Report on the Safety Assessment of *p*-Phenylenediamine was published in the *Journal of the American College of Toxicology*. The Panel recognized that *p*-Phenylenediamine is a known sensitizer and some persons may be sensitized under intended conditions for use. For those persons not sensitized, the Expert Panel concluded that *p*-Phenylenediamine is safe as a hair dye ingredient at the current concentration of use.

2006 – Re-review of *p*-Phenylenediamine published in the *International Journal of Toxicology*. The Panel reaffirmed the original conclusion in March 2004.

June 2006 - The Panel reviewed an expanded grouping of Phenylenediamine ingredients. The Panel rejected the large grouping and called for the ingredients to be divided into smaller groupings. Discussion was tabled so that the Phenylenediamines could be reorganized into 5 subsections.

June 2007 – The Panel discussed the status of the Phenylenediamine ingredients following a presentation by Industry. The Panel determined that the large grouping of Phenylenediamine ingredients should be broken out into individual reports.

September 2007 – The Panel reviewed the grouping of *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate for the first time. The Panel issued a Tentative Amended Report with the conclusion that these 3 ingredients are safe as cosmetic ingredients in the practices of use and concentrations described in the safety assessment.

December 2007 – The Panel issued a Final Amended Report with the conclusion that *p*-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate are safe as hair dyes in the practices of use and concentration described in the safety assessment.

2022-2023 – Review of the available published literature since 2007 was conducted in accordance to CIR Procedures regarding re-review of ingredients after ~15 years. Because the document was not published in the *International Journal of Toxicology* after the Panel finalized the Amended Report in 2007, the document is being presented to the Panel as a Draft Amended Report and is incorporating the data from that report along with newly available data.

December 2023 - The Panel issued a Tentative Amended Report for public comment with the conclusion 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 the safety assessment.

***p*-Phneylenediamine and Salts Data Profile* – June 2024 – Christina Burnett**																															
				Toxicokinetics			**Acute Tox**			**Repeated Dose Tox**			**DART**		**Genotox**		**Carci**			**Dermal Irritation**			**Dermal Sensitization**				**Ocular Irritation**		**Clinical Studies**	
	Reported Use	**Method of Mfg**	**Impurities**	**log P/log K_{ow}**	**Dermal Penetration**	**ADME**	**Dermal**	**Oral**	**Inhalation**	**Dermal**	**Oral**	**Inhalation**	**Dermal**	**Oral**	**In Vitro**	**In Vivo**	**Dermal**	**Oral**	**Other**	**In Vitro**	**Animal**	**Human**	**In Vitro**	**Animal**	**Human**	**Phototoxicity**	**In Vitro**	**Animal**	**Retrospective/ Multicenter**	**Case Reports**
p-Phenylenediamine; CAS No. 106-50-3	X O	O	X	X	X O	X O	X O	X O	X	X O	X O		X O	X O	X O	X O	X O	X	X		X O	O		X O	X O	O		X O	X O	X O
p-Phenylendiamine HCl; CAS No. 624-18-0	X O		X		X	X	O				O				X O	X O	X	X O	X											
p-Phenylenediamine Sulfate; CAS No 50994-40-6	O			X																X										

* “X” indicates that new data were available in a category for the ingredient; “O” indicates data that were previously reported.

p-Phenylenediamine, *p*-Phenylenediamine HCl, and *p*-Phenylenediamine Sulfate

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	EU	NTIS	NTP	FEMA	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
p-Phenylenediamine	106-50-3	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
p-Phenylenediamine HCl	624-18-0	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
p-Phenylenediamine Sulfate	16245-77-5 50994-40-6	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Search Strategy (from 2000 on)

PubMed

(("p-phenylenediamine") OR (106-50-3[EC/RN Number]) – 1604 hits
Limited with "reproduction"– 19 hits, 4 relevant
Limited with "acute toxicity" – 39 hits, 15 relevant
(("phenylenediamine HCl") OR (624-18-0[EC/RN Number]) – 522 hits
(("phenylenediamine sulfate") OR (16245-77-5[EC/RN Number]) OR (50994-40-6[EC/RN Number]) – 623 hits

ECHA

Dossier available for p-Phenylenediamine (CAS No. 106-50-3) and p-Phenylenediamine Sulfate (CAS No. 16245-77-5; listed as benzene-1,4-diammonium sulphate)
No dossier available for p-Phenylenediamine HCl (CAS No. 624-18-0; listed as benzene-1,4-diamine dihydrochloride

LINKS

Search Engines

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)

appropriate qualifiers are used as necessary
search results are reviewed to identify relevant documents

Pertinent Websites

- wINCI - http://webdictionary.personalcarecouncil.org
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives

Last updated April 2024

- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
    - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

DECEMBER 2023 PANEL MEETING - INITIAL REVIEW OF DRAFT AMENDED REPORT

Belsito's Team Meeting – December 4, 2023

DR. BELSITO: If not, let's on to para-phenylenediamine. A lot of reading for nothing. Do not reopen.

DR. HELDRETH: And a lot of work for Christina.

DR. BELSITO: Christina, you've got your hand up.

MS. BURNETT: Hold on a second. We are reopening it, though, because the 2007 report was never published, correct?

DR. HELDRETH: That's right.

MS. BURNETT: So, this has to go forward in the process, but your conclusion remains the same, correct?

DR. BELSITO: I guess so, yeah.

MS. BURNETT: Just so that my year-long worth of work doesn't go away.

DR. BELSITO: Okay.

DR. HELDRETH: Right. Right.

DR. BELSITO: Just a few things on the document. On PDF page 18, I think the word 'which' is missing here. It says para-phenylenediamine is oxidized to produce quinone diamine, which reacts with a coupling agent.

DR. HELDRETH: Sure.

DR. BELSITO: It's quinone diamine and reacts with a coupling agent. So as currently phrased it would be the para-phenylenediamine that reacts with the coupling agent, not the quinone diamine. So, is it the quinone that reacts or is it the para-phenylenediamine.

DR. RETTIE: It's the quinone.

DR. BELSITO: So, you need the word 'which' there.

DR. HELDRETH: Easy enough.

DR. BELSITO: And, oh. So, we can still use the VCRP for now and that disappears when?

DR. HELDRETH: Well, the VCRP is no longer a live database as of February of this year but the --

DR. BELSITO: So, for these documents we were able to use it, but for June's -- or March's we won't.

DR. HELDRETH: I think we can continue to use the VCRP data that we have from this year until Cosmetics Direct goes live and we have new mandatory reported data to use because otherwise we're stuck in a stall.

DR. BELSITO: Okay. And then the use in eye makeup preparations, that will be in the Discussion as well, right? And should it be in the conclusion because now we're seeing reports of it being used to dye eyebrows and eyelashes? Or since it's not allowed by the FDA it's not worth putting in our conclusion?

DR. HELDRETH: That's right. We don't need to conclude on unallowed uses. Certainly covered in the Discussion, but.

DR. BELSITO: I would put it in the Discussion only because it is being used and that's why we haven't looked at its safety for those uses because it's not allowed by our FDA.

DR. HELDRETH: Right.

DR. BELSITO: Because Europe is looking at it.

DR. RETTIE: So, under impurities back up a little bit, there's a sentence about the process not lending itself to the formation of aminobiphenyl compounds. I kind of wondered, well I guess back maybe last time we looked at this aminobiphenyl was a compound people worried about, but I just wondered if we could delete that from the impurities.

DR. BELSITO: What PDF page are you on, Allan?

DR. RETTIE: Nineteen.

MS. BURNETT: It's in the italicized information from the original report.

DR. RETTIE: I wondered if it was relevant. Maybe Tom really wanted that in last time. I don't have a problem leaving it there, it just doesn't seem to flow that well when I read it.

DR. BELSITO: I mean, oftentimes we just carry (audio skip).

DR. RETTIE: Sure. This is italic so that's just what you had in last time. And it's okay to leave it there.

DR. BELSITO: On PDF page 20, Christina -- is it 20? Yeah, the paragraph above non-cosmetic use, that paragraph, the second line says no concern regarding systemic toxicity to use of para-phenylenediamine oxidative hair dyes.

MS. BURNETT: Mm-hmm.

DR. BELSITO: I guess I put something missing, but should it be from use of para-phenylenediamine oxidative hair dyes.

MS. BURNETT: I will go back and check and correct the wording as needed.

DR. BELSITO: Okay. And then we later on mentioned black henna tattoos but they're not mentioned under non-cosmetic use.

MS. BURNETT: I'm sorry. What was that again?

DR. BELSITO: Under non-cosmetic use we don't -- subsequently in the report we discussed about para-phenylenediamine being used in black henna tattoos and the FDA reacting to that.

MS. BURNETT: Sure.

DR. BELSITO: But we don't mention that at all in non-cosmetic uses. Is that because of the FDA's actions or?

MS. BURNETT: No, I can add something there.

DR. BELSITO: Yeah.

DR. HELDRETH: I mean, tattoos fall within cosmetic use.

DR. BELSITO: Oh, really?

DR. HELDRETH: Mm-hmm. Tattoos are considered cosmetics in the U.S. Even though FDA has said they have not approved a single color for injection into the skin. So, there are no approved uses for the colorants in tattoos.

DR. KLAASSEN: So, should we be reviewing them?

DR. BELSITO: Well, we certainly know they're used, and they cause horrific, in some individuals, because the percentage of PPD is as high as seven or eight percent in some of these black henna tattoos and I've seen kids end up with keloidal scars in the shape of the octopus they had put on their arm on a beach. I mean, the reactions are that horrific that they can leave -- particularly people who are prone to it -- long-term scars. I mean, it definitely should be banned in black henna tattoos and --

DR. HELDRETH: I mean, it's an unapproved use at this point.

DR. BELSITO: Right. But do we mention it at all, or should we mention it? I mean, what do we say about them because there is something that we do say here later on in the -- yeah. Under clinical studies, risk of para-phenylenediamine in henna tattoos, that's clinical study.

MS. BURNETT: On PDF Page 32 are the case reports related to tattooing if that's what you're looking for.

DR. BELSITO: Yeah. And the FDA established a reporting hotline. Only 70 cases. I've had more than 70 cases. And the AAD endorsed a ban on the practice of applying. So maybe it's worth in the discussion saying something about our opinion of its use in black henna tattoos.

DR. HELDRETH: Yeah. We could repeat the U.S. FDA has determined that the uses of para-phenylenediamine other than as a hair dye are unapproved including use in dark black henna tattoo products and then say something about the Panel agrees that this is --

DR. BELSITO: Yeah. I would do that, Christina. Make me feel happy.

MS. BURNETT: Okay.

DR. BELSITO: And for our discussion we also have now negative repro-tox that we can add to that prior discussion. Okay.

DR. RETTIE: Just a little clarification on page 21 under dermal penetration. We say an octanol/water partition coefficient of 0.5 and so then we report it as the log in Table 2. I just wondered if we should put in parenthesis after 0.5 log P equals minus 0.3 to gel with the table.

DR. HELDRETH: We can do that.

DR. RETTIE: It's a little thing.

DR. BELSITO: Okay. Anything else or are we all set here?

DR. SNYDER: I think we're all set.

Cohen's Team Meeting – December 4, 2023

DR. COHEN: Okay, let's move onto para-phenylenediamine. So, this was a draft amended report for para-phenylenediamine, p-phenylenediamine hydrochloride, and para-phenylenediamine sulfate. In 1985 the panel published a safety assessment for PPD with a conclusion that PPD is a known sensitizer in some person's may be sensitized under it's intended conditions of use. For those persons not sensitized, the panel concluded that PPD 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 and it's been more than 15 years.

According to the 2023 VCRP, PPD is reported to be used in at least 200 formulations, the majority of them being hair colors. However, uses have been reported in eye makeup -- seven of them -- so this goes beyond the purview of this panel because these exemptions for these color additives are for hair color, not for cosmetic use.

We have only one reported use for PPD hydrochloride in a hair coloring shampoo and no uses for the sulfate. For concentration of use, PPD had a maximum concentration of three percent in hair dyes with a maximum on head concentration of one percent and no concentration of use were reported with the salts. And then we have two margins of safety calculations performed by SCCS. One which was a conventional calculation, the other was a toxicokinetic based approach and whereas do we agree with these calculations, or should we develop our own?

So, there's a lot there and I'll just open it up for discussion for PPD. Susan, you want to start?

DR. TILTON: Sure. So, just some of the new toxicity data show that it had low dermal absorption, rapid metabolism after oral exposure. And in this case, we also had some mixed in vitro genotoxicity. In this case, though, it seemed to not necessarily be consistent with or without metabolic activation but there was no genotoxicity in vivo, there was also no carcinogenicity.

There was some evidence of ocular irritation and then it was also not irritating dermally but sensitizing in multiple studies. So, in this case I felt like we had some insufficiencies in terms of evaluating for use in eye products, primarily we don't have concentration of use information or detail about how it's used in eye products.

DR. COHEN: Well, I might interject here. It's not supposed to be used there, right? So, these seven uses in a cosmetic are -- am I being hyperbolic by saying illegal use? Unpermitted use of the product. So, we're only discussing this in the context of a hair dye and one of the comments I wanted to have is, you know, we say this is not within the purview of the panel, I think we should make a stronger statement that these are not permitted, right? These are just now --

DR. BERGFELD: You put it in the discussion.

DR. COHEN: Yeah.

DR. BERGFELD: You put it in the discussion.

MS. FIUME: It currently does state the use is not permitted under the cosmetic use section. The second to last sentence of the second paragraph.

DR. COHEN: Second to last sentence. Under use, right?

MS. FIUME: Under use, yeah. It says, "p-phenylenediamine is an unapproved color additive in cosmetic products and thereby such use is not permitted."

DR. COHEN: Yeah.

MS. FIUME: And then it says, "the use is not with purview of the panel."

DR. COHEN: Yeah. So, Susan, if the insufficiency is related to that we wouldn't need to go down that road.

DR. BERGFELD: Except I would pull it out again in the discussion because I think it's important.

DR. COHEN: I completely agree. I'm saying, but I wouldn't issue an IDA for that.

DR. BERGFELD: No.

DR. COHEN: I'm sorry I interrupted you, Susan, I just didn't want to start an IDA on that.

DR. TILTON: No. No, that's helpful. So, otherwise, in terms of its use as a hair dye ingredient, based on the data that we have I would approve as safe as a hair dye ingredient up to three percent.

DR. COHEN: Concentration for hair on -- I think I would leave percentage out and just discuss it in the context of the report.

DR. TILTON: Okay.

DR. COHEN: You know what I mean? Because it's different in different circumstances. David?

DR. ROSS: Yeah, and I would just comment on that one that the maximum that I had was one percent. For the most part I agree with what Susan has been saying. When I looked at this, dermal looked okay, oral moderately toxic, DART was okay, genotox. Mixed in vitro but, again, negative in vivo. Little irritation dermally. Strong sensitizer but it's coal tar so we don't have to look at that. The ocular was mild, as Susan said, although the SCCS, I think, had a limit on ocular. Yeah. Not for eyelashes/eyebrows, that's coal tar exemption.

DR. COHEN: Yes.

DR. ROSS: Eyelash use at two percent. Professional use only. So, they had something -- but I agree with Wilma, we could probably deal with that in the discussion, right, and do that. The margin of safety I think we should keep both of them. There was a question about do we need one or the other and do we do an additional CIR or our own margin of safety because we have a one percent on hair. My recommendation would be no because we don't have an absorption study of one percent. You would be making some inaccurate assumptions there. So that was my take of it.

So, I didn't have anything, David. Surprisingly for me, I thought this could probably go. So.

DR. COHEN: David, were the two margins of safety are the calculations correct? I know that's presumptuous perhaps, but.

DR. ROSS: They looked correct to me. I mean, we got 200 which is still presumption of safety that's greater than a hundred. That's the conventional one and then you've got a toxicokinetic one which what that basically does is it compares the areas into the curve. You know, so you've got this area and then you move to the experimental and you compare the areas under the curve, and you actually like to see about a 25 on that. They had a 23.3 and they thought well, that was okay, SCCS, because it's a hair dye, it's not used every day. So, I thought both were okay.

DR. COHEN: Tom?

DR. SLAGA: I agree with both Susan and David.

DR. COHEN: Okay. One question. On the margin of safety calculation what do they use? They're using -- how much exposure? Is it like 50 percent of the head or -- the area of exposure?

DR. ROSS: Yeah, that's in the SCCS guidelines. We used to use, I think, 700 and then the -- and just off the top of my head -- no pun intended -- I think it's about 565 now. I mean, it's documented in SCCS. I can pull it up if you want.

DR. COHEN: No, 566 what?

MS. BURNETT: Yeah. The margin of safety on the first paragraph says skin area surface of 580 centimeters squared.

DR. ROSS: I was close, Christina.

MS. BURNETT: Yep, yep, yep, very close. Rounding works.

DR. COHEN: So, I guess the question is just a practical one. If someone has long hair and they've put this in, there will be a component of neck and back exposure -- at least temporarily while it's sitting there -- has that ever been factored in? I know it's supposed to be up here, like it's all done like that. Okay.

DR. BERGFELD: David, they are caped and toweled so it doesn't happen.

DR. COHEN: Except when you're doing it at home, right?

DR. BERGFELD: Well, maybe.

DR. COHEN: Okay. So, I think we have a safe as used. Right?

DR. BERGFELD: I think so.

DR. COHEN: That's not mine tomorrow anyway. But. Okay. Hold on. Let's move on.

Full Panel Meeting – December 5, 2023

DR. BELSITO: In 1985, the Panel published a safety assessment on p-Phenylenediamine with a conclusion that it's a known sensitizer. Some persons may be sensitized under intended concentrations of use. For those persons not sensitized, we concluded that p- Phenylenediamine is safe as a hair dye at the current concentrations of use. The conclusion was reaffirmed in 2006, and in 2007 we issued a Final Amended Report that included p-Phenylenediamine, dihydrochloride and sulfate salts of p-Phenylenediamine. And, because it's been more than 15 years, we're being asked to review the safety of p-Phenylenediamine, p-Phenylenediamine Hydrochloride and p-Phenylenediamine Sulfate.

There were tons of data that were added that were all new, but no substantive change that would indicate that these materials are not safe as currently used in hair dyes. And, so, our conclusion is to not reopen the document.

DR. BERGFELD: Is there a second or a comment?

DR. COHEN: Isn't this a Draft Amended Report?

DR. HELDRETH: Yes. And the previous version of this report for some reason it never was published. So if we just continue forward with this one then there's no published report out there from the past.

DR. BELSITO: So, then we will continue forward with a conclusion that the Expert Panel concluded that p-Phenylenediamine, p-Phenylenediamine Hydrochloride, and p-Phenylenediamine Sulfate are safe as hair dyes in the practice of use and concentrations as described in the safety assessment.

DR. COHEN: Second.

DR. BERGFELD: Seconded. Any further discussion, edits?

DR. BELSITO: Yeah, basically we can copy the discussion that we had previously.

DR. BERGFELD: Yeah.

DR. BELSITO: It would be an addition that we now have negative reproductive toxicity data, and wanted to add in that discussion that it's not for use in black henna tattoos. Because apparently the FDA considers tattoos cosmetic, so to some extent that may be in our purview. I don't know. But I think it needs to be made clear that we find it would be inappropriate for use in a henna tattoo.

DR. COHEN: Agreed.

DR. BERGFELD: Agreed. Any other discussion, or add to the Discussion area of the document? Seeing none, I'll call the question. All those opposed? Abstaining? Unanimously approved as safe, with addition of the edits or the Discussion the items.

DECEMBER 1983 PANEL MEETING – FIRST PUBLIC REVIEW OF P-PHENYLENEDIAMINE

Full Panel

The Expert Panel discussed the carcinogenicity, mutagenicity and teratogenicity data for this ingredient and found them to be negative. Dr. Schroeter stated the toxicity of this compound is minimal, but clinically it does produce sensitization.

At the behest of industry, Dr. McEwen provided the Panel with copies of a proposed safety conclusion, which read as follows:

"The CIR Expert Panel notes that PPD (or 2-NPPD and 4-NOPD [another report discussed at the same meeting]) is a sensitizer; however, available data on this ingredient as used in hair coloring products indicates that the warnings and directions for use mandated by section 601 of the US Food, Drug and Cosmetic Act, give reasonable protection from adverse dermatologic effects for consumers. With the data on other potential hazards considered also, the Panel concludes that PPD (or 2-NPPD and 4-NOPD) is safe as currently used in cosmetic products."

To support this conclusion, Dr. McEwen provided the Panel with copies of the North American Contact Dermatitis Group's "Prospective Study of Cosmetic Reactions: 1977-1980," as well as cosmetic adverse reaction data received by FDA directly from consumers. Both of these demonstrated that hair dye-related contact dermatitis was relatively low. He also provided the Panel with human repeat insult patch test data on 206 patients, supporting industry's contention that the sensitization risk from hair dyes is slight. Dr. Bergfeld disputed these studies on the basis of the test population used. Dr. Boutwell suggested the conclusion be amended to reflect the fact that the ingredient is safe for nonsensitized individuals, but is nevertheless a sensitizer for some persons.

Mr. Eiermann provided the correct wording for the exemption of coal tar hair dyes from the principal adulteration and color additive provisions (Sections 601 and 706) of the Federal Food Drug and Cosmetic Act of 1938 when their label bears a cautionary statement and patch test instructions. The correct wording is already present in the 2-NPPD/4-NOPD document.

Upon motion by Dr. Berndt, seconded by Dr. Schroeter, the following conclusion was unanimously approved:

"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 concentration of use."

Dr. Bergfeld suggested that determinations as to whether the material tested was an irritant or a sensitizer be stated in the results of patch tests (Table 11).

Subject to this and other minor revisions, the document will be announced as a Tentative Report.

[NO FURTHER MINUTES FROM PUBLIC DISCUSSIONS ON P-PHENYLENEDIAMINE PRIOR TO THE 1985 PUBLICATION ARE AVAILABLE.]

MARCH 15-16, 2004 PANEL MEETING – RE-REVIEW OF PHENYLENEDIAMINE

Team Minutes Not Available

Full Panel

A CIR Final Report with the following conclusion was published in 1985: 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.

Dr. Belsito stated that many new studies on *p*-Phenylenediamine appear in the published literature, and that these data do not warrant a change in the Panel's conclusion on this ingredient. However, he noted that *p*-Phenylenediamine is increasingly appearing in henna tattoos, and that individuals who are *p*-Phenylenediamine-sensitive are not aware of this.

Dr. Belsito said that when the Panel reaffirms its Final conclusion on *p*-Phenylenediamine, which indicates that it is safe as a hair dye for those persons not sensitized, it should also be noted that its leave-on use in temporary henna tattoos is considered unsafe based on the sensitization risk.

Dr. McEwen said that the use of *p*-Phenylenediamine in henna tattoos is a concern. He added that the manner in which this concern should be addressed needs to be considered, because, under the current law, these are not cosmetic products that would be allowed in the United States.

Dr. Belsito said that there must be some way in which the Panel may alert the public as to the presence of *p*-Phenylenediamine in henna tattoos. He recalled one of his patients (allergic to *p*-Phenylenediamine) who received a henna tattoo and developed a severe allergic reaction on the upper back that resulted in keloidal scarring.

Dr. McEwen recommended a separate statement, apart from the Panel's re-review.

Dr. Katz said that the use of *p*-Phenylenediamine in henna for tattoos is not a legal use in this country; neither *p*-Phenylenediamine nor henna is approved for tattoo use. Dr. Katz added that FDA's website has information for consumers indicating that *p*-Phenylenediamine is found in some cases of black henna to make the color of the henna black, and emphasized that FDA can only reach a certain group of consumers.

Dr. Katz said that FDA tries to get the word out that the use of *p*-Phenylenediamine and henna in tattoos is not a legal or legitimate use of either dye. She said that it would also be useful for consumers or any organization that gathers information from consumers with problems or reactions to report any adverse reactions to FDA. She added that the more reports that FDA receives, the more likely that FDA potentially will be able to look into the issue to determine exactly what can be done with regard to enforcement.

Dr. Katz also encouraged the dermatologists on the Panel to get the word out to colleagues to report patients with an allergic reaction, due to use of the color, to FDA.

Regarding the Panel's issuance of a letter to address a particular concern, Ms. Weintraub wanted to know whether this type of letter has ever been accompanied by a press release that could be posted at the CTFA or CIR website.

Ms. Weintraub expressed concern that a letter would not be disseminated widely and noted that there should be some type of proactive action.

Dr. Andersen said that CIR has never used a press release vehicle. However, when the Panel decided to terminate its review of Methyl Methacrylate and support FDA's position (as captured on FDA's website), the Panel's discussion was captured on CIR's website.

Similarly, Dr. Andersen said that FDA's position on the use of *p*-Phenylenediamine in henna in tattoos has the Panel's support and that the Panel also supports the reporting of adverse events to FDA.

Ms. Weintraub remarked that a very specific, limited population would visit the CIR or CTFA website, and that some type of action that would be more proactive is preferred.

Dr. Andersen wanted to know which other websites should be used.

Dr. McEwen recommended that the warning regarding the use of *p*-Phenylenediamine in tattoos be posted at CFA's website and that CIR should be cited.

Ms. Weintraub said that she would solicit input from Dr. Andersen, Dr. McEwen and the Panel in generating the statement.

The Panel unanimously concluded that the Final Report on *p*-Phenylenediamine should not be reopened.

Dr. Marks recommended that the discussion section of the re-review contain the Panel's revised patch test instructions for hair dyes as well as the revised hair dye epidemiology statement.

JUNE 12-13, 2006 MEETING – 1ST REVIEW OF PHENYLENEDIAMINE HAIR DYE GROUP

Discussion on 4,6-Bis(2-Hydroxyethoxy)-m-Phenylenediamine HCl; N,N'-Bis(2-Hydroxyethyl)-2-Nitro-p-Phenylenediamine; 2-Chloro-5-Nitro-N-Hydroxyethyl p-Phenylenediamine; N,N-Diethyl-p-Phenylenediamine Sulfate; N,N-Dimethyl-N-Hydroxyethyl-3-Nitro-p-Phenylenediamine; N,N-Dimethyl-p-Phenylenediamine; 2,6-Dimethyl-p-Phenylenediamine; 2,6-Dimethyl-p-Phenylenediamine HCl; N,N-Dimethyl-p-Phenylenediamine Sulfate; 4-Ethoxy-m-Phenylenediamine Sulfate; 4-Fluoro-6-Methyl-m-Phenylenediamine Sulfate; Hydroxyethyl-p-Phenylenediamine Sulfate; Hydroxypropyl Bis(N-Hydroxyethyl-p-Phenylenediamine) HCl; N-Methoxyethyl-p-Phenylenediamine HCl; 2-Methoxy-p-Phenylenediamine Sulfate; N-Methyl-3-Nitro-p-Phenylenediamine; 4-Nitro-o-Phenylenediamine Dihydrochloride; 2-Nitro-p-Phenylenediamine Dihydrochloride; 4-Nitro-o-Phenylenediamine HCl; 4-Nitro-m-Phenylenediamine Sulfate; 4-Nitro-o-Phenylenediamine Sulfate; 2-Nitro-p-Phenylenediamine Sulfate; PEG-3 2,2'-Di-p-Phenylenediamine; p-Phenylenediamine HCl; and p-Phenylenediamine Sulfate.

Belsito Team – June 12, 2006

Dr. Belsito recommended the preparation of a table (i.e., checklist table) that includes a listing of the studies a on each ingredient that are included in the safety assessment, considering that the safety assessment involves a large group of ingredients. The table will not be part of the report, but actually, a handout that will allow the Panel to easily determine the availability of data on each ingredient. The organization of the table will incorporate the Hair Coloring Technical Committee's suggestions for ingredient groupings that are outlined in the memorandum (dated 2/9/06) that was received from industry.

Dr. Klaassen acknowledged that data used in the development of SCCNFP opinions on Phenylenediamines are also included in the CIR draft report, but that a statement to this effect does not need to precede each summary of data (see page 55) referenced in an SCCNFP report.

Dr. Belsito's Team agreed that the next report draft should reflect the ingredient groupings that are recommended in the memorandum from industry.

Dr. Andersen suggested that the draft report should not be divided into separate reports, based on industry's recommendations for ingredient groupings, but, rather, that the existing draft report should be reorganized to include subheadings for each ingredient group that is included in the memorandum.

Dr. Belsito's Team agreed that the draft report on Phenylenediamines should be tabled, pending reorganization of the report into the various ingredient groups. It was also agreed that a checklist table (includes a listing of the studies a on each ingredient that are included in the safety assessment) will be provided to the Panel prior to the next review of the draft report.

Marks Team – June 12, 2006

It was agreed that the draft report needs to be reorganized, based on the memo from Dr. John Bailey recommending that the ingredients (and studies associated with each) should be organized into five separate groups. Chemical structures should be included at the beginning of each section, followed by use frequency and use concentration data, toxicity data, etc.

Dr. Bergfeld noted that PEG-3, 2, 2'-Di-p-Phenylenediamine is very toxic to the skin. She also said that the Panel needs to consider deleting some of the case reports that are repetitive.

It was noted that hair dye regulations should appear at the end of the report, rather than at the end of each section.

Drs. Bergfeld and Shank noted that the data from Eastman Kodak Co. Do not contain the test concentrations or animal models.

Regarding page 54 of the draft report, it was noted that actual test concentrations are not included and that the animal strains tested are not indicated.

It was noted that a CIR final report on N,N-Bis (2-Hydroxyethyl)-p-Phenylenediamine Sulfate was published in 1992 and, thus, that data on this ingredient should be diluted from the table on ingredient use frequencies and use concentrations in the draft report.

Dr. Marks said that it should be noted in the report Introduction that N,N-Bis (2-Hydroxyethyl)-p-Phenylenediamine Sulfate has been reviewed by the Panel.

Dr. Bergfeld said that CTFA should be asked to provide a list of Phenylenediamine ingredients that may be included in the International Cosmetic Ingredient Dictionary and Handbook under different naming conventions.

Dr. Andersen stated that p-Phenylenediamine should be added to the draft report because the CIR final report on this ingredient will become the master document.

Dr. Marks' Team agreed that the draft report on Phenylenediamines should be tabled pending reorganization of the report into a document that is more manageable.

Full Panel – June 13, 2006

Dr. Belsito stated that, prior to this Panel meeting, a memorandum from Dr. John Bailey suggesting that the report be reorganized was received. The recommendation involves dividing the current report into the following five individual reports: (1) Addendum to the CIR report on p-Phenylenediamine, to include the two salts (2) Addendum to the CIR report on 2-Nitro-p-Phenylenediamine and 4-Nitro-o-Phenylenediamine, to include their salts, (3) A report on the remaining direct dyes (all contain a nitro group), (4) A report on the remaining m-Phenylenediamine dyes, and (5) A report on the remaining p-Phenylenediamine dyes.

Dr. Belsito said that his Team considers this to be a logical request, in terms of looking at groups where data on one chemical would allow the Panel to better understand potential toxicologic risks that may be associated with other chemicals. He added that his Team is not in favor of dividing the current report into five separate reports, because data on p-Phenylenediamine and p-Phenylenediamine salts, and, also, the o- and m-Phenylenediamines, would be important in terms of evaluating the safety of ingredients in some of the other groups.

Dr. Belsito said that his Team recommends that the Draft Report on the Phenylenediamines be tabled, pending reorganization of the report into five sections (one per ingredient group). The development of a table indicating the types of data that are available on each hair dye in the five ingredient groups was also recommended. This table will be provided as a handout to assist the Panel in determining the availability of data on each ingredient.

The Panel voted unanimously in favor of tabling the Draft Report on Phenylenediamines, pending reorganization of the report into five sections (one per ingredient group).

Dr. Slaga confirmed that each of the five sections in the report will be complete, from beginning to end. He then noted that 4-Nitro-m-Phenylenediamine Sulfate is included in Group 3, and wanted to know why it is not included in Group 2.

Dr. Belsito wanted to know why 4-Nitro-m-Pyhenylenediamine is not included in Group 4.

Dr. McEwen said that the ingredients are listed on the basis of their chemistry and how they are being used. He noted that the way in which the meta-Phenylenediamines are being used is different from that of the ortho-Phenylenediamines.

Dr. McEwen asked for clarification that all of the information on p-Phenylenediamine that exists now will be incorporated into the first section of the Draft Report (i.e., Group 1 ingredients), and noted that the same applies to the Nitro-Phenylenediamines relative to Group 2. He added that when the revised Draft Report is completed, this document will supercede previous CIR reports. This means that, at that point in time, it will no longer be necessary to consult previous CIR reports on Phenylenediamines for information.

Dr. Andersen said that the concept of including all of the data on Phenylenediamines from previous CIR reports on these ingredients in a single document would create a useful reference document.

Dr. Marks wanted to make certain that N,N-Bis(2-Hydroxyethyl)-p-Phenylenediamine Sulfate will be included in the revised document, because this ingredient is included in over 400 cosmetic products.

Dr. Belsito said that the International Cosmetic Ingredient Dictionary and Handbook should be consulted to make sure that Phenylenediamines are not missing from the Draft Report.

Dr. McEwen said that CIR's cumulative list of safety assessments should also be checked to make sure that all of the Phenylenediamines on this list are included in the Draft Report as well.

Dr. Andersen said that it is not expected that the revised Draft Report will be ready for consideration at the August Panel meeting.

Dr. Bergfeld announced that the Draft Report on Phenylenediamines is being tabled.

JUNE 2007 MEETING – 2ND REVIEW OF PHENYLENEDIAMINE HAIR DYE GROUP

Team Minutes Not Available

Full Panel – June 5, 2007

Dr. Bergfeld stated that a presentation on Phenylenediamine hair dyes was given on the preceding day. She noted that the presentation focused on reclassification of the dyes by type and use. Drs. Bergfeld and Andersen agreed that the presentation was very enlightening.

Dr. Andersen provided a sense of how the Panel should proceed based on the information that was presented. He noted that there are groups of Phenylenediamine hair dyes that can be combined with their salts and reviews could be conducted as the groups are formed. In many cases, these groups will also include other Phenylenediamines for which data will be available, as submitted to the European authorities for their hair dye reviews.

Dr. Andersen said that there are also Phenylenediamine hair dyes that are not in current use or suspected not to be in current use. Regarding these, the idea was that there would be very little productivity in conducting reviews because data will not be provided.

Dr. Andersen said that the other point that was made is that there is likely little ability to extrapolate the toxicity of one Phenylenediamine with different side groups to other Phenylenediamines with dramatically different side groups. This fact alone argues that separate reports need to be prepared. Thus, Dr. Andersen noted that this pattern will be followed as CIR moves forward with reviews on Phenylenediamines. He added that these reviews will be developed for the Panel's consideration as soon as possible, and that at least one report will be included on the agenda for the September 24-25, 2007 Panel meeting.

Dr. Bergfeld said that given the comments and new epidemiology studies that will become available and the Panel's position statements, this information needs to be organized in a way that would facilitate the Panel's review. With this done, it would be possible for the Panel to continually access this information easily for future reviews on hair dyes.

SEPTEMBER 2007 MEETING – 1ST REVIEW OF P-PHENYLENEDIAMINE AND ITS SALTS

Team Minutes Not Available

Full Panel

Tentative Amended Final Report - safe conclusion, unanimous: The CIR Expert Panel concluded that p-Phenylenediamine, p-Phenylenediamine HCl, and p-Phenylenediamine Sulfate are safe as cosmetic ingredients in the practices of use and concentrations described in this safety assessment.

Dr. Marks: In 1985, the Panel issued a safe report on p-Phenylenediamine. In 2004, this safety assessment was not reopened and the Panel reconfirmed that safe conclusion. Now, we are in a position to add salts procedurally.

Dr. Belsito: We discussed in our group how to handle, when we are integrating this report, information that we have gotten that sort of updates what was in the old report. Specifically, the information that we were given as to how p-Phenylenediamine can be manufactured and how that differs from what previously was in the report.

Our Team felt that the old manufacturing information that we had should simply be deleted from the new report. The updated methods of manufacture, i.e., the 3 specific ways that p-Phenylenediamine can be manufactured (presented to us yesterday), should replace the old information in the text. We should then go on to point out that it is the assumption of the Panel that cosmetic grade p-Phenylenediamine is made by the benzene nitration, without chlorination, which results in a final product that does not contain chlorinated compounds such as chloro- and dichloro- anilines or aminobiphenyls.

Dr. Marks: The conclusion that is in the published final report needs to be changed so that it conforms to the current format.

The boilerplate relating to ingredients reviewed in a safety assessment that are not currently being used that was discussed during the Panel's deliberations on the safety of the PPG Methyl Ethers needs to be included in the discussion section of this

report, considering that it is the Panel's understanding that p-Phenylenediamine HCl and p-Phenylenediamine Sulfate are not being used in cosmetics.

There was a concern relating to 4-aminobiphenyl. Dr. Shank will elaborate.

Dr. Shank: The following statement should be included in the Discussion: 4-Aminobiphenyl, a known urinary bladder carcinogen, has been found in research grade 1,4-phenylenediamine. [The reference for this is Turesky et al. 2003]. Cosmetic grade p-Phenylenediamine should not contain 4-aminobiphenyl.

The following correction should be made in the second paragraph of the discussion. Currently, the second sentence reads as follows: Phototoxicity and photosensitization data are not available. But, in fact, we do have such data.

The sentence should be revised as follows: Phototoxicity and photosensitization data are limited, but suggest that sensitization is about the same with or without UV light. [Dr. Shank noted that the studies that he is referring to are on pages 116 and 117. - He must mean pages 118 and 119.]

Dr. Belsito: Statements regarding the hair dye epidemiology boilerplate should be removed from the discussion.

Dr. Marks: There are a couple of articles reporting cases of depigmentation after exposure to p-Phenylenediamine. We would suggest that we group those articles together and, in the Discussion, they should be addressed by saying: Clinically, this has to be a very uncommon or rare event and not a significant safety concern.

Dr. Belsito: We did the same thing and actually went a bit further in the discussion: It was not clear whether this was vitiligo induced by an allergic reaction, true chemical leukoderma, or post-inflammatory, and the nature of this reaction requires further study.

Dr. Belsito: As you said also, it is a very rare event.

Dr. Bergfeld confirmed that all of the skin depigmentation case reports will be grouped under a Skin Depigmentation subheading in the section on Case Reports.

Dr. Snyder: CTFA will be providing the Panel with a memo with data regarding the DuPont method of manufacture since they are the sole source. He wanted to make sure that it is in the record that the Panel is supposed to receive this.

Dr. Shank: Is DuPont the sole source for U.S.- made cosmetics or fo all dyes?

Dr. Julie Skare: Dupont is the current global supplier of p-Phenylenediamine.

Dr. Bergfeld: We will put a reminder here to get that from you so that we have a reference source in house.

DECEMBER 2007 MEETING –2ND REVIEW OF P-PHENYLENEDIAMINE AND ITS SALTS

Team Minutes Not Available

Full Panel

Final Report - unanimous

Dr. Belsito: The same change in the hair dye epidemiology statement.

Dr. Andersen: In listening to the various discussions yesterday, I think that there is an ongoing concern about the data from the FDA Center for Toxicologic Research that looked at actual hair dye products and found 4-aminobiphenyl in the products. We need to have discussion elements that address the potential concern.

Subsequently, I understand that you learned from DuPont, the major supplier of p-Phenylenediamine, that the product that is sold to cosmetics companies is 99% pure p-Phenylenediamine, which, in that same Turesky study from NCTR, had no 4-ABP as part of it. All of this needs to be presented in the study.

I would argue along with the Panel's expression of an expectation that the industry is using the 99% grade product. Just in case there is someone out there who doesn't get it, this is the Panel's expectation and it is OK because we have the data.

Dr. Bailey: The editorial changes that were talked about yesterday will be incorporated into the text, as it will be released after the Panel votes.

Dr. Andersen: The other issue that we need to address is what we heard from industry yesterday, which is that there may be some further editorial comments, as technical experts from industry look at this document.

I don't see that as a problem as long as the conclusion does not change. So, the actual issuance of this may not be until after the 1st of the year. I will certainly alert the Panel if there is anything substantive.

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

Status: Draft Final Amended Report for Panel Review
Release Date: May 10, 2024
Panel Meeting Date: June 3-4, 2024

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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
MEL	measured exposure level
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
NESIL	no expected sensitization induction level
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 April 2024. 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]

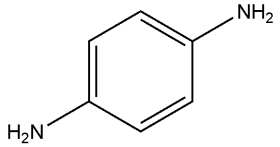


Figure 1. *p*-Phenylenediamine

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*-phenylene-diamine, 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 included herein were obtained from the FDA's Voluntary Cosmetic Registration Program (VCRP) database in 2023 (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) in 2022 (maximum use concentrations). The data were provided by cosmetic product categories, based at that time 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 these ingredients 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.

These ingredients are considered coal tar hair dyes 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 because it is a potent contact allergen.

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.

TOXICOKINETIC STUDIES

p-Phenylenediamine is absorbed and excreted by both animals and humans.[2] In a study with [^{3}H]p-Phenylenediamine HCl in rabbits, a biphasic clearance of radioactivity from the blood was observed after intravenous administration, with half-life values of 24 min and 43.5 h . When applied dermally in rabbits, only 0.05% of the applied radioactivity could be detected in the blood after 20 min. Radioactivity was distributed throughout the body and in the blood after the intravenous and topical administration of p-Phenylenediamine HCl to mice. In dogs, p-Phenylenediamine HCl 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 free base and dihydrochloride salt were applied topically to humans; 12.7% of the radioactivity from the free base and 14.8% from the salt were recovered in the urine within 5 d, respectively. 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 [^{3}H]p-Phenylenediamine HCl 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-phenylene-diamine (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. Receptor fluid was normal saline solution. 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 2.4×10^{-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). Cutaneous activities for MAPPD formation ranged from 0.41 - 3.68 nmol/mg/min, and DAPPD formation ranged from 0.65 - 3.25 nmol/mg protein/min. Similar results were obtained with keratinocytes.

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 μg_{eq}h/ml and 27.30 μg_{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.

Other 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 of radioactivity 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.

Occupational Studies

In a study of 18 hairdressers (3 males and 15 females), the subjects were exposed to oxidative hair dyes under controlled conditions for 6 workdays.[26] The 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. Three separate phases of hair dyeing were monitored: dye preparation/hair dyeing, rinsing/shampooing/conditioning, and cutting/drying/styling. Urine and blood analyses were performed on all subjects. Adverse events not reported in any subjects. Hair dye residues in the hair + scalp of the training heads accounted for 53.46 ± 4.06% of the applied radioactivity, while the hair wash accounted for 45.47 $\pm$ 2.95%, the waste and protective equipment worn by the hairdressers accounted for 0.41 $\pm$ 0.16%, and the dye mixing bowls accounted for 2.88 $\pm$ 0.54%. Concentration of dye in the plasma of the hairdressers was below the limit of quantification (< 10 ng *p*-Phenylenediamine$_{eq}$/ml). Total urinary excretion of radiolabel from the hairdressers ranged from a total of < 2 to 18 µg *p*-Phenylenediamine$_{eq}$, and was similar in subjects exposed during the 3 different phases of hair dyeing The mean mass balance of radiolabel for the 6 d study was 102.50 ± 2.20%, and overall mean total systemic exposure of hairdressers to oxidative hair dyes during a workday that included 6 hair dyeing processes was estimated to be < 0.36 µg *p*-Phenylenediamine$_{eq}$/kg bw/work d.

A hand rinse study was performed to assess skin exposure to permanent hair dye compound in 33 hairdressers (13 males and 20 females).[27] Hand rinse samples were collected from each hand before the start of hair dyeing, after application of the dye, and after cutting newly-dyed hair. The left and right hands were simultaneously rinsed in a polyethene bag containing 50 ml rinsing solution (0.2 M ascorbic acid in borate buffer with 10% ethanol), and the hairdressers were instructed to shake their hands vigorously for 2 min before the rinse liquids were collected in bottles. Half of the hairdressers (16 subjects) did not use gloves during application of the dye, and none wore gloves for cutting hair. Samples were analyzed for pertinent aromatic amines and resorcinol using HPLC. Of the 54 hair dyes used, 10 contained *p*-Phenylenediamine. After the application step, *p*-Phenylenediamine was 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 from background contamination. Exposure loading for *p*-Phenylenediamine was 22 - 989 nmol/hand.

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 cm^2 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] One of 2 rats treated at 75 mg/kg died while a single rat treated at 50 mg/kg survived in a study in which rats 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 in the skin, 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 (concentration not reported).

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 in the blood 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.[28] 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 in which *p*-Phenylene-diamine was applied topically twice weekly throughout growth, mating, gestation, and lactation phases at up to 4% in oxidative formulation.[29] In a 90-d dermal study in male rats painted daily 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 (NOEL/NOAEL not identified).[30] 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.[31] 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.[5,32-35] *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.[36-40] Mixed results were observed in additional bacterial strain genotoxicity studies with up 5 mg/ml *p*-Phenylenediamine.[5,41,42] 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.[32,33] 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;[43,44] 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.[37] *p*-Phenylenediamine was not genotoxic in an unscheduled DNA synthesis assay in rat hepatocytes at up to 1 µmol/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.[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.[37]

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).[45] 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

p-Phenylenediamine HCl

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.

CARCINOGENICITY STUDIES

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] 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.

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-Phenylenediamine 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.

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.[46] 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.[36]

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,[5,28] 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.[5,9,47] 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.[36] 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 *p*-Phenylenediamine 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 placental glutathione S-transferase positive foci when compared to controls in a 5 wk intragastric study.[48,49]

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-Phenylene-diamine, 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-Phenylene-diamine 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.[32,33] 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.[50] 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.[51] Notable toxicity to the lymphocytes was observed at 10 ppm and greater.

The effect of 40 mg/ml *p*-Phenylenediamine dissolved in 1 ml DMSO on proliferation, lactate dehydrogenase (LDH) levels, apoptosis, and subsequent mRNA levels of caspase-3 was studied in human HK-2 proximal tubular epithelial cells.[52] The proliferation of HK-2 cells was significantly inhibited ($p < 0.01$) by *p*-Phenylenediamine, with or without hydrogen peroxide. The level of apoptosis of HK-2 cells, the mRNA levels of caspase-3 and LDH production were significantly increased following stimulation by *p*-Phenylenediamine ($p < 0.01$) compared to controls or cells treated by solely with hydrogen peroxide. A typical apoptotic morphological change was observed under electron microscopy in response to *p*-Phenylenediamine.

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.[53] 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.[50] 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).[54] 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), 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-Phenylenediamine in propylene glycol.[2] No further details provided.

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.[5,7,55] (The guinea pig LLNA was determined not to be reliable by ECHA due to use of guinea pig instead of mouse, lack of positive control, and lack of study details). 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%.[5,56] *p*-Phenylenediamine was sensitizing in predictive studies in human subjects when tested at up to 1% in pet.[57]

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.[51] 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.[51]

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% of a hair dye formulation containing *p*-Phenylenediamine (concentration in formulation not reported) daily for up to 3 mo; it should be noted that this study was rated as not reliable by ECHA (reason for rating not stated).[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.[58] 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.[59] 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.[60] 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.[61] 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.[62] 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 from over the last 40 years are summarized in Table 11. Sensitization to *p*-Phenylenediamine has been observed around the globe, with sensitization rates in patients with suspected allergic contact dermatitis varying greatly, independent of region or span of time.[63-98] 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%.[80] In Europe, a German multicenter study reported a positivity rate of only 1.5% in 1994 to 1995.[69] In both of these studies, it was not known if the patients were already determined to have an allergy to hair products or hair dyes. In contrast, 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).[89,92] However, these studies were conducted in patients with known or suspected allergy to hair products or hair dyes.

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.[99-112] 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.[113-121]

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.[122-140] 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.[141] 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.[142]

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.[143] 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.[144] 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.[145] 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.[146] 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).[72]

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.[147] 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.[148] 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.[149] 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.[150] 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.[151] 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; allergic responses were to nickel-sulfate hexahydrate, ammonium thioglycolate, and 4-chloro-3,5-xylenol).[152] 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.[92] 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.

Effects Observed with Occupational Exposure

The results of occupational exposure studies, mainly involving hairdressers, are summarized in Table 14.[26,27,72,95,153-157] In studies of around 300 hairdressers, the frequency of reactions to *p*-Phenylenediamine was reported to be around 17 to 20% and frequency of reactions to *p*-Phenylenediamine HCl was reported to be nearly 8%, while in a study with 3095 positive reactions to *p*-Phenylenediamine, 8.3% of reactions were occupationally related and 72.8% of those reactions occurred in hairdressers/cosmetologists.[72,153,156] 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.[158,159] 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.

A study on the occupational exposure of hairdressers to hair dyes that are associated with the development of allergic contact dermatitis reported a no-expected-sensitization-induction-level (NESIL) of 27.5 µg/cm² for *p*-Phenylenediamine.[160] In this research, the measured exposure level (MEL) of *p*-Phenylenediamine was quantified by summing the amounts of radio-labeled [^{14}C]*p*-Phenylenediamine found in the stratum corneum, epidermis, dermis, and receptor fluid. The hair dye product used in the study contained 2% *p*-Phenylenediamine after being mixed with the developer, aligning with the maximum allowable use concentration in the European Union. The dose of hair dye product applied to human skin was 445.6 ± 39.2 µg/cm² per application. The results further indicated that estimated hairdresser hand exposure level is 2.7-fold below the individual NESIL (at 27.5 µg/cm²) for *p*-Phenylenediamine.

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 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. With regard to the reported use in eye makeup preparations, the US Federal 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. *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.

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 2% (free base) in oxidizing hair dyes. Additionally, *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. Additionally, the SCCS found that *p*-Phenylenediamine in hair dyes remains a considerable concern for consumer safety because it is a potent contact allergen.

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 an occupational ADME study in 18 hairdressers over 6 workdays, the majority of the radioactivity from application of 2% [^{14}C]*p*-Phenylenediamine to training heads was accounted for in the hair + scalp of the training head (53.46%). No adverse effects were observed in the hairdressers. The mean mass balance of radiolabel for the 6 d study was 102.50 %, and overall mean total systemic exposure of hairdressers to oxidative hair dyes during a workday that included 6 hair dyeing processes was estimated to be < 0.36 µg *p*-Phenylenediamine$_{eq}$/kg bw/workday. In a hand rinse study in 33 hairdressers, exposure loading for *p*-Phenylenediamine was 22-989 nmol/hand. Hairdressers' skin is found to be exposed to allergenic compounds during hair dyeing, with exposure occurring during application, cutting, and from background contamination.

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. One of 2 rats treated at 75 mg/kg died while a single rat treated at 50 mg/kg survived in a study in which rats 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. In an inhalation study, 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 guinea pig study of a hair dye formulation that contained *p*-Phenylenediamine (concentration not reported).

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 applied topically twice weekly throughout growth, mating, gestation, and lactation phases at up to 4% in oxidative formulation. In a 90-d dermal study in male rats painted daily 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 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. Assays in CHO cells indicated a dose-related increase 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, guinea pig lymph node cells, and human HK-2 proximal tubular epithelial 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% of a hair dye formulation containing *p*-Phenylenediamine (concentration in formulation not reported) daily for up to 3 mo (study was rated as not reliable by ECHA). 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 in patients with suspected allergic contact dermatitis 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. In Europe, a German multicenter study reported a positive rate of only 1.5% in 1994 to 1995. In both of these studies, it was not known if the patients were already determined to have an allergy to hair products or hair dyes. In contrast, 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). However, these studies were conducted in patients with known or suspected allergy to hair products or hair dyes. 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. In studies of around 300 hairdressers, the frequency of reactions to *p*-Phenylenediamine was reported to be around 17 to 20% and frequency of reactions to *p*-Phenylenediamine HCl was reported to be nearly 8%, while in a study with 3095 positive reactions to *p*-Phenylenediamine, 8.3% of reactions were occupationally related and 72.8% of those reactions occurred in hairdressers/cosmetologists. 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 study on the occupational exposure of hairdressers to hair dyes associated with the development of allergic contact dermatitis reported a NESIL of 27.5 $\mu g/cm^2$ for *p*-Phenylenediamine.

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 dose-dependent toxicity and a NOAEL of 8 mg/kg/d in a 90-d oral study, the negative results in developmental and reproductive toxicity and carcinogenicity studies, the acceptable margin of safety values, 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, use in eye makeup products 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. However, use of the ingredients in this report as direct hair dyes is not reported.

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]

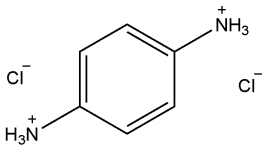
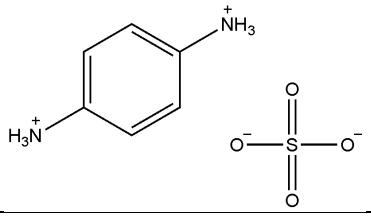
Ingredient & CAS No.	Definition	Function(s)
p-Phenylenediamine 106-50-3	*p*-Phenylenediamine is the aromatic amine that conforms to the structure in Figure 1.	hair colorant
p-Phenylenediamine HCl 624-18-0	*p*-Phenylenediamine HCl is the aromatic amine salt that conforms to the structure: 	hair colorant
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
Molecular Weight (g/mol)	206.22	161
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	water	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; the only rat treated with 100 mg/kg died within 90 min of dosing and 1 rat in 75 mg/g died within 175 min; the single rat that received 50 mg/kg survived; 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 (no further details provided); 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 in the blood 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]

Table 5. Repeated dose toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
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; histopathological observations of the liver and kidney showed no remarkable changes in the treated groups or the control except for fatty degeneration in the liver of males and females in the high dose group	[28]
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	[29]
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	[30]
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, particularly in the 50 mg/kg dose group; 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 in degeneration, apoptosis, and increased ROS levels	[31]

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; not mutagenic without metabolic activation to TA98 or with or without metabolic activation to TA100	[35]
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	[33]
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; mutagenicity was not observed in TA98NR without metabolic activation or in TA100NR with metabolic activation.	[32]
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	[34]
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	[41]
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	[42]

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	33
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	32
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	Not genotoxic	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	36
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; no mutagenicity was observed in the remaining strains with or without metabolic activation	37
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 (no further details provided); not possible to determine whether test substance was mutagenic in *E.coli* strain	40

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	[38]
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	[39]
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	[43]
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	[44]
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	[37]
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	[37]
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	[45]

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 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 mixture (1:1 ratio) applied topically (0.5 ml) to shaved back skin once per 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; 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	[36]
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 weight gains 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 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	[28]

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 on 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	[47]
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 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	[36]

Table 8. Carcinogenicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
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	[49]
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 S-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 S-transferase positive foci when compared to controls	[48]

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]

Table 9. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
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
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%	55

Table 9. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
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]
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 due to guinea pigs being used in place of mice, no positive controls were used, and lack of study details	[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	[56]
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	[57]
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.	[57]

Table 10. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
ANIMAL						
Hair dye formulation containing *p*-Phenylenediamine; purity not reported	not reported	not reported	groups of 10 rats; no further details provided	5, 10, or 15% hair dye formulation instilled into 1 eye once daily for up to 3 mo; no further details provided	Keratitis and corneal opacities observed; no further details provided. This study was rated as not reliable by ECHA.	[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; after 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	Known Allergy to Hair Product and/or Hair Dye?	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	Unknown	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	[66]
3515	Multicenter study of patients tested at NACDG clinics using standardized patch testing technique with 52 allergens that included 1% *p*-Phenylenediamine	Unknown	North America	July 1, 1992 to June 30, 1994	6.3% (527) patients had positive reaction to *p*-Phenylenediamine; 47.3% had present relevance and 19.4% had past relevance	[65]
3111	Multicenter study of patients tested at NACDG clinic using standardized patch testing technique with 49 allergens that included 1% *p*-Phenylenediamine pet.	Unknown	North America	July 1, 1994 to June 30, 1996	6.8% of patients had positive reaction to *p*-Phenylenediamine; 6.7, 21, 20, and 14% had a definite, probable, possible, or past relevance, respectively	[63]
5831	Multicenter study of patients tested at NACDG clinic using standardized patch testing technique with 50 allergens that included 1% *p*-Phenylenediamine pet.	Unknown	North America	July 1, 1998 to December 31, 2000	4.9% of patients had positive reaction to *p*-Phenylenediamine; 5.9, 22.2, 26.4, and 18.1% had a definite, probable, possible, or past relevance, respectively	[64]
36,491	Multicenter study of patients tested at IVDK with standard series, including 1% *p*-Phenylenediamine pet.	Unknown	Germany and Austria	1990 to 1995	5.0% (crude rate, 4.8% standardized for age and sex) had positive reactions to *p*-Phenylenediamine	[67]
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	Unknown	Germany	between 1994 and 1995	1.5% of patients overall had positive reaction to *p*-Phenylenediamine	[69]
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.	Suspected	Germany, Austria, and Switzerland	January 2013 to December 2020	31.6% (580 reactions) of consumers had positive reactions to p-Phenylenediamine	[95]
			Retrospective Studies			
54,917	Retrospective study of individuals tested to 1.0% *p*-Phenylenediamine pet. by the NACDG	Yes	North America	1994 to 2018	5.6% (3095) of patients had positive reaction to *p*-Phenylenediamine; over half (55.3%) of reactions were ++ or +++ at final reading and 60.9% were of current relevance	[72]
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	Unknown	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	[76]
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.	Yes	North America	2001 to 2016	35.8% (1524) of patients had reaction to *p*-Phenylenediamine	[70]
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.	Unknown	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	[80]
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	Unknown	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)	[83]
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.	Unknown	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	[78]

Table 11. Multicenter and retrospective studies

# Patients	Clinical Testing Type	Known Allergy to Hair Product and/or Hair Dye?	Location	Years	Results	Reference
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.	Unknown	United States	January 2011 to December 2020	6.1% (9 reactions) of patients had positive patch test reactions to *p*-Phenylenediamine	[77]
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)	Suspected	United States	2015 to 2022	10% (6 reactions) of patients had positive reactions to *p*-Phenylenediamine	[97]
2686	Retrospective study of patients patch tested with Mayo clinic standard series; *p*-Phenylenediamine was tested at 1% in pet.	Unknown	United States	2017 to 2021	5.2% (141 reactions) of patients had positive reaction to *p*-Phenylenediamine	[79]
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.	Unknown	North America	January 2019 to December 2020	5.6% (231 reactions) of patients had positive reactions to *p*-Phenylenediamine; 2.6, 33.8, 26, and 22.5% had a definite, probable, possible, or past relevance, respectively	[71]
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 yr; British Contact Dermatitis standard series including 1% *p*-Phenylenediamine in petrolatum; Finn chambers for 48 h	Unknown	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	[73]
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.	Unknown	London, UK	October 2016 to April 2018	8.3% (13 reactions) of patients had positive reaction to *p*-Phenylenediamine; 1.3 and 7.1% had definite or probable current relevance and none had possible or past relevance	[98]
826	Study of late patch test reactions of at least 10 d after application; ICDRG test guidelines using Finn chambers; 1% *p*-Phenylenediamine pet.	Yes	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)	[81]
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)	Unknown	Valencia, Spain;	May 1991 to May 2019	5.5% (11) of patients had positive reactions to *p*-Phenylenediamine	[68]
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.	Unknown	Spain	2004 to 2014	4.1% (386 reactions) of patients had positive reaction to *p*-Phenylenediamine	[82]
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%	Yes	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	[92]
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)	Suspected	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); 90.5% were of current clinical relevance	[74]
1309	Retrospective study of patch test results of patients with suspected contact allergies; European baseline series, included 1% *p*-Phenylenediamine pet.	Unknown	Turkey	2013 to 2019	3.7% (48 reactions) of patients had positive reactions to *p*-Phenylenediamine	[85]

Table 11. Multicenter and retrospective studies

# Patients	Clinical Testing Type	Known Allergy to Hair Product and/or Hair Dye?	Location	Years	Results	Reference
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)	Unknown	Turkey	2015 to 2019	2.61% (5 reactions) of patients had positive reaction to *p*-Phenylenediamine	[96]
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)	Unknown	Saudi Arabia	January 2012 to February 2015	22.9% (17 reactions) of patients had positive reactions to *p*-Phenylenediamine	[93]
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	Unknown	India	2010 to 2014	5% (5 reactions) of patients had positive patch test reactions to *p*-Phenylenediamine	[91]
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	Suspected	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	[90]
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	Unknown	India	dates not reported	9.2% (14 reactions) of patients had positive reaction to *p*-Phenylenediamine	[88]
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	Suspected	India	dates not reported	67.5% (54 reactions) of patients had positive reaction to *p*-Phenylenediamine, another 3 patients had irritant reactions	[89]
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)	Unknown	Sri Lanka	2012 to 2018	12.3% (54 reactions) of patients had positive reactions to *p*-Phenylenediamine	[94]
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.	Yes	Thailand	2009 to 2018	6.4% (182) of patients had positive reactions to *p*-Phenylenediamine	[87]
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.	Unknown	Singapore	November 2007 to October 2017	13.4% (399) of patients had positive reactions to *p*-Phenylenediamine	[86]
5865	Retrospective study of patients patch tested with the Japanese baseline series, including *p*-Phenylenediamine	Unknown	Japan	April 2015 to March 2019	8.9% of patients had positive reactions to *p*-Phenylenediamine	[84]
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	Unknown	New Zealand	2008 to 2020	4.9% of patients had positive reaction to *p*-Phenylenediamine	[75]

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, papular 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	[99]
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	[103]
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*-Phenylenediamine 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	[100]
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	[101]
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	[102]
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*-Phenylene-diamine in petrolatum except one that was ++ at 0.1%; simultaneous positive patch reactions observed to IPPD and local anesthetics	[104]
61-yr-old male	Pruritic eruptions on upper back; several months prior, patient had recurring pruritic sensation of scalp and pruritic eruptions on the scalp 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	[106]
74-yr-old female	Erythematous, scaly and pruritic rash of scalp with associated hair loss and erythematous papular 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 incident.	Patch test was +++ to *p*-Phenylenediamine, ++ to methylchloroisothiazolinone, and + to carba mix and gold	[107]
63-yr-old male	Patient presented with elevated liver enzymes (ALT and AST), but otherwise asymptomatic and other laboratory test results normal; patient was under stable treatment for human immunodeficiency virus, was immune to hepatitis A and B viruses, had negative test results for hepatitis C and E viruses, and no prior history of liver disease or cirrhosis and had no other risk factors for viral hepatitis or fatty liver disease. Ultrasound findings were negative; a liver biopsy found 50 to 60% fat with features of steatohepatitis and moderately severe fibrosis without cirrhosis. Over the course of several months, the patient's liver enzymes continued to rise. Patient mentioned previously starting to use a coloring shampoo that contained *p*-Phenylenediamine that caused a severed skin reaction to his legs and arm. After the patient was advised to discontinue products, liver enzyme levels dropped by 3-fold in 2 mo.	Patient was not tested for allergy to *p*-Phenylenediamine	[112]
42-yr-old female	Patient presented with a history of a severe rash after using a hair dye; for 2 yr prior, patient had been using hair rinses every 6 - 8 wk. In the month prior to the severe reaction, patient had dyed her hair and noted mild transient scalp itching; when she subsequently used the same hair dye and within 2 h of application, her scalp was red and weeping and she developed an eczematous rash involving the periorbital area, forehead, ears, cheeks, sides of neck that progressed to involvement of trunk and buttocks; with medical treatment, the rash eventually cleared after 4 mo.	An immediate urticarial reaction to1% *p*-Phenylene-diamine observed 20 min after application of an occluded patch test; an eczematous reaction developed on the patch site 48 h later	[111]

Table 12. Case reports related to hair dye use

Patient(s)	Presentation	Patch Test Results	Reference
57-yr-old female	Syncope occurred within minutes of using a hair dye that contained *p*-Phenylenediamine; patient also developed nausea, vomiting, and difficulty breathing with a cold sweat. Patient had used same preparation for 8 yr without incidence, but then started to have incidents of itchy bumps on the head and hands 10 - 30 min after dye applications that resolved within 3 h.	Patch tests were positive for patient's hair dye and *p*-Phenylenediamine. Patient had an immediate-type allergy to *p*-Phenylenediamine with no signs of delayed typed allergic reaction	[110]
43-yr-old female	Patient reported immediate pruritus of the scalp, with a malaise that lasted for 15 min, following hair dye application; the patient then had generalized pruritus and erythema, dyspnea, vomiting and hypotonia. Symptoms disappeared after 2 h and contact dermatitis appeared thereafter, lasting 8 d. The patient previously experienced dermatitis of the scalp and neck after coloring hair every 6 wk; no previous use of temporary black henna tattoos reported.	With open tests applied for 30 sec and read after 20 min, the results were doubtful for the hair dye and negative for the coloring cream and developer tested separately. Another open test where the material was applied for 20 min and then read yielded positive results for the hair dye and negative for the coloring cream, developer, and the individual components. Open test for 20 min with oxidized 2,4-diaminophenoxyethanol-HCL was positive. In patch tests read after 72 h, 1% *p*-Phenylenediamine pet. and 1% toluene-2,5-diamine pet. were ++	[109]
57-yr-old male	African American patient with immunodeficiency virus presented with scaling, edematous, focally excoriated, hypopigmented, and slightly erythematous papules on cheeks, postauricular areas, and back of neck appeared 2 d after application of hair dye containing *p*-Phenylenediamine; no involvement of scalp observed; facial rash occurred once before within 24 to 48 h after application, but relationship to hair dye discounted by patient since scalp was not involved;	Patch test with 1% *p*-Phenylenediamine on right forearm produced mild pruritus with erythema tiny papules at patch test at 48 h; 6-d reading showed +++ reaction with erosions	[108]

Table 13. Case reports related to dermal hair dye and 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	[137]
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	[138]
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	[122]
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	[139]
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	[127]
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	[132]
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	[126]
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	[131]
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	[129]
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	[130]
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	[134]
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	[123]

Table 14. Assessment of effects in persons occupationally exposed to *p*-Phenylenediamine

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	[153]
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	[155]
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	[156]
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	[72]
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*-Phenylenediamine; 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. Renal effects were also associated with exposure to henna.	[157]
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	[95]
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	[154]

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6

Final Report on the Safety Assessment of *p*-Phenylenediamine

p-Phenylenediamine is a cosmetic hair dye intermediate used in permanent hair coloring products at concentrations of up to 5 percent (diluted 1:1 with an oxidizing agent prior to application). The extensive animal toxicity test data on p-Phenylenediamine and permanent cosmetic hair dyes containing this compound show that the degree of toxicity varies with concentration, test system and animal species. Animal data support a conclusion that this compound is neither a teratogen nor a carcinogen. Epidemiological data also support that hair dyes containing this ingredient are not carcinogenic. p-Phenylenediamine is a sensitizer and some persons may be sensitized under intended conditions of use.

For those persons not sensitized, it is concluded that p-Phenylenediamine is safe as a hair dye ingredient at the current concentrations of use.

INTRODUCTION

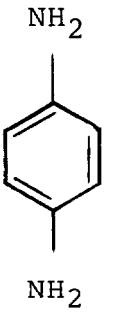
p-Phenylenediamine is reviewed in this report; it is used only in permanent hair dyes. 2-Nitro-*p*-phenylenediamine and 4-nitro-*o*-phenylenediamine are reviewed separately; these compounds are used in permanent and semipermanent hair dyes. All three compounds and other hair dye ingredients may be combined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

CHEMISTRY

Definition and Structure

p-Phenylenediamine (PPDA) (CAS No. 106-50-3) is the aromatic amine that

conforms to the formula[1]:



Other names for PPDA include: 1,4-phenylenediamine; 1,4-benzenediamine; *p*-benzenediamine; 4-aminoaniline; *p*-aminoaniline; 1,4-diaminobenzene; para-diaminobenzene; PPD; BASF Ursol D; Benzofur D; Developer PF; Durafur Black R; Fouramine D; Fourrine 1; Fourrine D; Fur Black 41867; Fur Brown 41866; Furro D; Fur Yellow; Futramine D; Nako H; Orsin; Pelagol D; Pelagol DR; Pelagol Grey D; Peltol D; Renal PF; Santoflex LC; Tertral D; Ursol D; Zoba Black D; Rodol D; C.I. 76060; C.I. Developer 13; and C.I. Oxidation Base 10.[1-7]

Method of Manufacture and Impurities

PPDA has been produced commercially in the US for more than 50 years.[4,8] It is prepared by reducing *p*-dinitrobenzene with iron and hydrochloric acid, or by reducing *p*-nitroaniline with either (1) iron and hydrochloric acid, (2) iron, ammonium polysulfide, and hydrogen, or (3) iron and ferrous chloride.[3,4,7,9] The compound can be purified by crystallization.[3]

PPDA is commercially available as the free base, which is slowly oxidized by air, and as the sulfate and hydrochloride salts, which are more stable.[10]

One technical grade of PPDA that is available in the US has the following specifications[4,11]:

Purity	99.2 percent minimum
Moisture content	0.1 percent maximum
o-Phenylenediamine	0.1 percent maximum
Iron	50 mg/kg maximum

In Japan, PPDA is available as a commercial grade with a minimum purity of 99.5 percent, a minimum melting point of 139°C, and 1-amino-4-nitrobenzene and 4-aminophenol as impurities. In the United Kingdom, PPDA has a minimum purity of 99 percent, and contains traces of 1-amino-4-nitrobenzene and 4,4'-diaminoazobenzene (DAAB).[4] (In a 60-week feeding study in mice, DAAB was not carcinogenic.[12]) Although there is no indication that these particular products are available in the US, imports of PPDA have been reported.[4,13]

US production and imports of PPDA have been estimated to total 36.5 to 48 million pounds per year.[13] The cosmetic ingredient safety analysis summary of PPDA provided to the Cosmetic Ingredient Review (CIR) by CTFA[10] states the major US manufacturer of PPDA produces 5 to 10 million pounds per year by

catalytic reduction of *p*-nitroaniline and that 90 percent of the production is used internally by the company. Approximately 90,000 pounds are used annually in the manufacture of hair dyes.

Chemical and Physical Properties

Aromatic amines, such as PPDA, are nonpolar bases that are readily converted to highly water-soluble hydrochloride salts. Whereas the salts of aromatic amines are relatively stable,[4] free aromatic amines are usually quite unstable to light, heat, and oxygen and oxidize to colored quinoneimines, quinones, and various polymerized products.[14] 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.[14]

Radomski[14] suggested that phenylenediamines and their oxidation products are highly reactive substances that would be expected to react with tissue nucleophiles, causing various biological effects. Several other authors have noted that 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.[15–17] Phenylenediamine compounds are also potent antioxidants.[18]

Data on the chemical and physical properties of PPDA are presented in Table 1. PPDA occurs in the form of white to light purple monoclinic crystals. It is solu-

TABLE 1. Chemical and Physical Properties

		Reference
Formula	$C_6H_8N_2$	25
Molecular weight	108.15	25
Boiling point	267°C	2–5,7,13,19,20,22,25,27
Melting point	139°C	2,20
	140°C	4,25,27,31
	141°C	22
	145–147°C	3,5,7,13,19
Solubility	Water: 3.8 percent at 24°C	20
Volatility (technical product)	<1 mm at 21°C	4,32
Vapor density	3.72	5
Flash point (closed cup)	155.5°C	3,5,19,22
Octanol/water partition coefficient (log P_{oct}/water)	−0.25	13,33
UV light absorption	λ max:246 nm (E_1^1 = 788), 315 nm (E_1^1 = 184)	4,25,27

ble in water, alcohol, ether, benzene, chloroform, and acetone and is insoluble in caustic soda. The compound reacts with oxidizing materials. On exposure to air, PPDA oxidizes to form a purple or black color. Brown and black colors can also develop when the compound is exposed to 5 percent $FeCl_3$ and 3 percent H_2O_2 solutions, respectively. Quinoneimine compounds resulting from the oxidation of PPDA may hydrolyze in aqueous media to yield *p*-benzoquinone and ammonia. PPDA is combustible and, when heated, emits highly toxic fumes of nitrogen compounds. Degradation following exposure to activated sludge microorganisms has also been reported.[2-5,7,19-25]

In addition to the UV absorption data presented in Table 1, data on the infrared,[26] mass,[27] and fluorescence[28] spectra of PPDA have been published.

Reaction Products from the Oxidation of PPDA

The oxidation of PPDA (Fig. 1A) by molecular oxygen results initially in the formation of *p*-benzoquinone diimine (Fig. 1B). The diimine may react to give either a polymer of the diimine (Fig. 1C) or Bandrowski's base (Fig. 1D). *p*-Benzoquinone diimine may also undergo hydrolysis to form monoimine (Fig. 1E), and then undergo further hydrolysis to *p*-benzoquinone (Fig. 1F) and its decomposition products (humic acid). Nitroaniline and 4,4'-diaminoazobenzene (DAAB) have also been identified as minor oxidation products. Other reaction products may be formed under certain conditions.[29,30]

The hydrolysis of *p*-benzoquinone diimine (Fig. 1B) to *p*-benzoquinone (Fig.

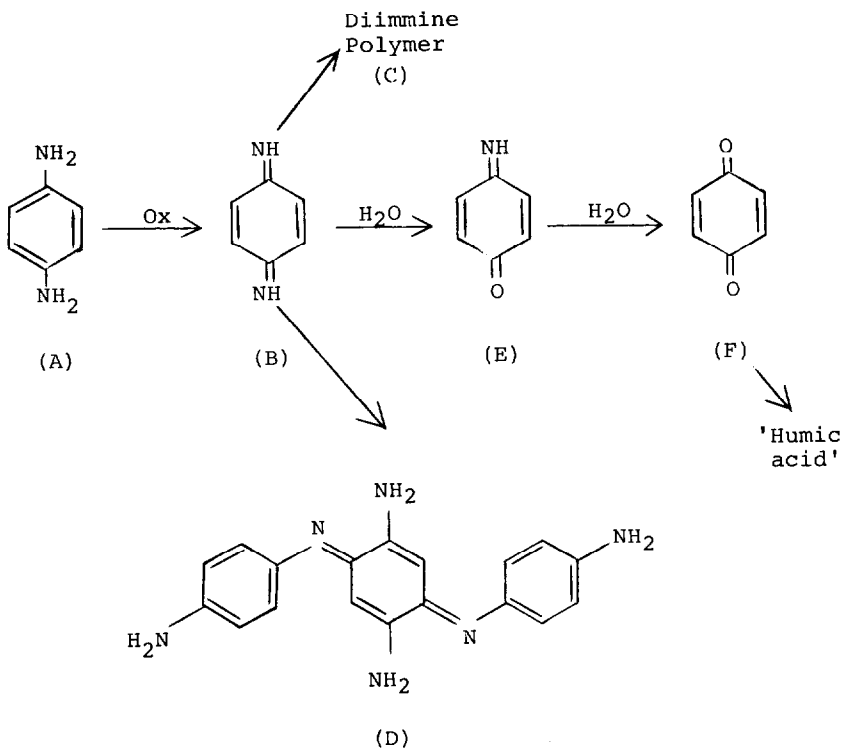


FIG. 1. Major products from the autooxidation of *p*-Phenylenediamine.[29,30]

1F) is only significant at a pH of less than 3 in the presence of a strong oxidizing agent, such as potassium ferricyanide, potassium dichromate, or ferric chloride. At a pH of greater than 9, the formation of *p*-benzoquinone (Fig. 1F) again is significant, but only if the PPDA solution is at a concentration of less than 0.001 percent. Polymerization of *p*-benzoquinone diimine (Fig. 1B) occurs at a pH greater than 9 when a PPDA solution ($>10^{-3}$ percent) is added to a solution of a strong chemical oxidant. The major reaction product under "most relevant conditions" (pH of 3 to 10 and PPDA concentrations of $>10^{-3}$ percent) is Bandrowski's base (Fig. 1D).[30]

Oxidative Hair Coloring Chemistry

In oxidative (permanent) hair coloring systems, the colored material is produced inside the hair fiber by oxidation of colorless intermediates.[30] To accomplish the color-forming reaction, three classes of chemical reactants are required: (1) the primary intermediates, (2) the oxidant, and (3) the couplers. Frequently employed intermediates are aromatic *o*- or *p*-diamines or aminophenols (Table 2).[34] The major primary intermediate used in the US for permanent hair dyes is PPDA.[35] Primary intermediates are capable of undergoing oxidation to form color benzoquinone imines, the "essential reactive species" in the color-forming reaction. The second necessary component is the oxidant. Hydrogen peroxide is the most frequently used oxidant, although various acids of solid organic hydrogen peroxide adducts are used depending on the hair dye product. Hydrogen peroxide is widely employed because it is a relatively unreactive oxidant and causes a slow oxidation of the primary intermediate in the dye bath. The third component necessary for color development is the coupler (Table 3). By virtue of their strong electron-donating groups, couplers react with the electrophilic quinone imines to produce leuko-indo dyes. When mixtures of couplers are used, the amount of each dye formed depends on (1) the relative concentration of the couplers in the dye bath, (2) the rate of coupler diffusion into the hair fiber, and (3) the relative reactivities of the couplers at the prevailing pH (Table 4).[34]

Some of the important color-forming reactions of PPDA are presented in Figure 2, and the chemistry of these reactions is summarized in Figure 3. The initial reaction involves oxidation of PPDA by the oxidant, or by oxygen formed by decomposition of the oxidant inside the hair fiber, to give *p*-benzoquinone diimine. Hair and skin act as catalysts in the oxidation process. *p*-Benzoquinone diimine in the form of its conjugate acid then reacts with the coupler and/or unoxidized

TABLE 2. Colors Produced by Primary Intermediates[36,37]

Compound	*Color on Hair*
p-Phenylenediamine	Dark brown
p-Toluylenediamine	Light reddish brown
p-Aminodiphenylamine	Dark gray-black
p-Aminophenol	Light auburn
2-Amino-5-hydroxytoluene	Golden blond
5-Amino-2-hydroxytoluene	Reddish blond
o-Aminophenol	Deep gold

TABLE 3. Colors Produced by PPDA in the Presence of Various Couplers[36,37]

Coupler	*Color in Hair*
None	Dark Brown
m-Phenylenediamine	Bluish purple
2,4-Diaminoanisole	Purple-blue
m-Aminophenol	Light brown
4-Methyl-3-aminophenol	Light brown
m-Methoxyphenol	Magenta
6-Methyl-3-aminophenol	Magenta
2,5-Xylenol	Bluish purple
Resorcinol	Greenish brown
Hydroquinone	Light gray-brown
Catechol	Gray-brown

TABLE 4. Reactivity of Commonly Used Couplers Toward *p*-Benzoquinone Diimine at 30°C and pH 9.5[34]

Coupler	*Experimental Second Order k**
Resorcinol	1.5×10^5
m-Aminophenol	5.5×10^4
2,4-Diaminoanisole	6.0×10^4
1-Naphthol	7.4×10^5
p-Phenylenediamine	34.7

*For d[dye]/dt = k[diimine] [coupler].

PPDA to yield a leuko-indo dye. Reaction occurs by electrophilic attack on the most nucleophilic site of the benzene ring of the coupler. If the reactive site on the coupler bears a methoxy group, the indo dye is formed nonoxidatively by elimination of methyl alcohol from the coupled intermediate. Some of these indo dyes are the final colored product in the hair, whereas others undergo further reaction to form polymeric indo compounds.[30,34-38]

Resorcinols react with *p*-benzoquinone diimine to give a green trinuclear dye and/or a brown polymeric indoaniline (Fig. 2).[34,39] *m*-Diamines couple with *p*-benzoquinone to yield blue 2-aminoindamines. Except for the methoxy derivatives, 2-aminoindamines have poor color stability and undergo intramolecular cyclization to red 2,8-diaminophenazines. *p*-Benzoquinone diimine couples with *m*-aminophenols at the position para to the hydroxy group to give magenta 2-aminoindoanilines, or, if this position is blocked, coupling occurs para to the amino group to yield magenta 2-hydroxyindamines. PPDA can also react with 2-aminoindoaniline to give a brown triangular dye. The magenta dyes are relatively unstable and fade to a brown species. Coupling of phenols with *p*-benzoquinone diimine yields purple indoanilines, whereas reaction of *p*-benzoquinone with *p*-diamines gives the brown dye, Bandrowski's base.[34]

Although the initial oxidation product of PPDA is usually *p*-benzoquinone diimine (with possible involvement of a free radical intermediate), the nature of the

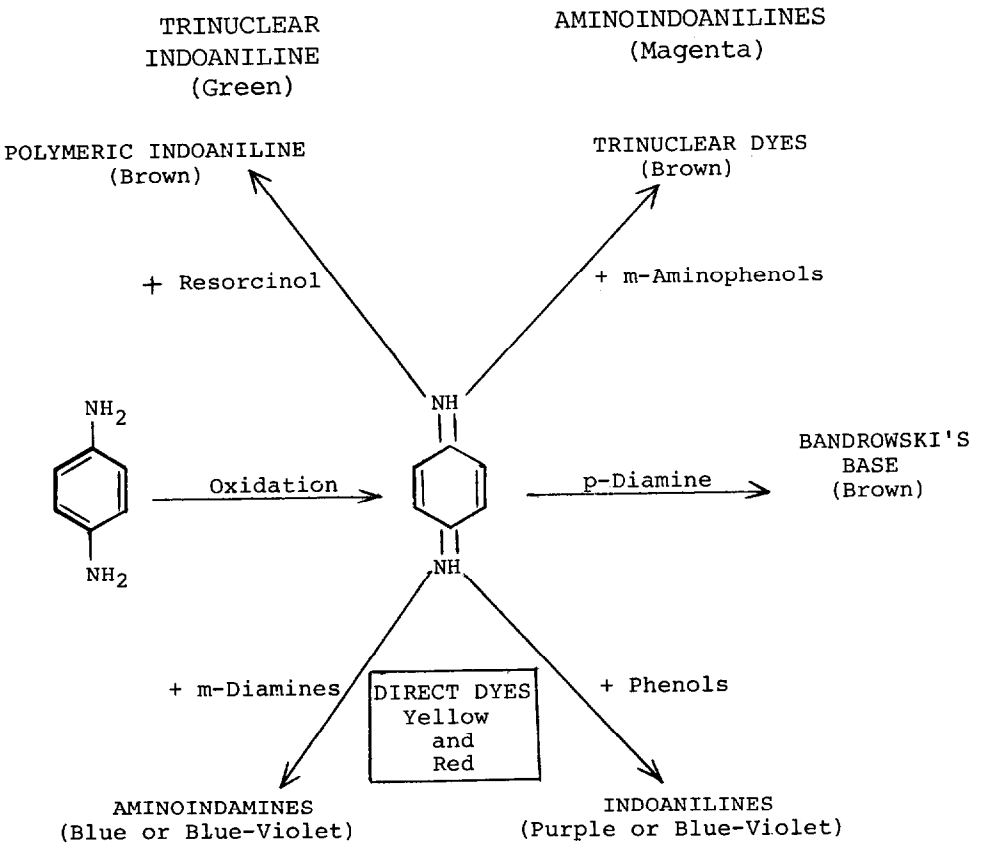


FIG. 2. Color-forming reactions in oxidative color development.[36,37]

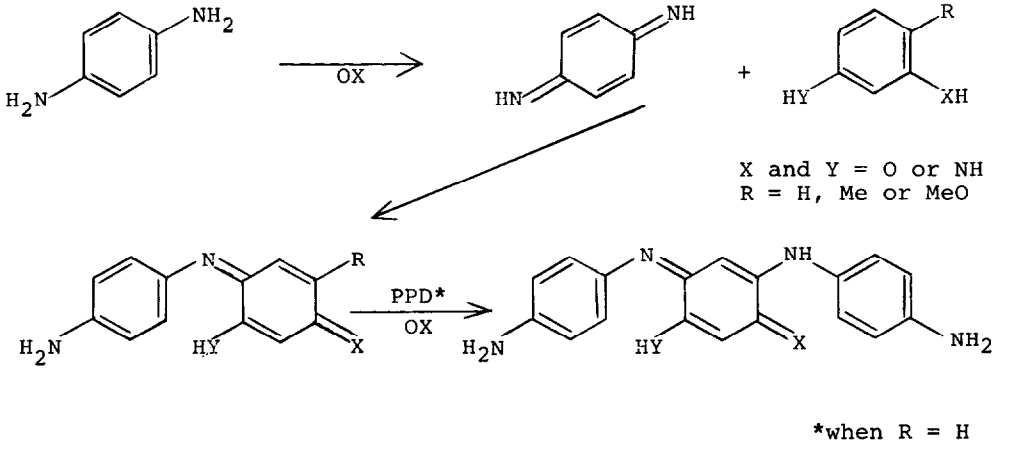


FIG. 3. The chemistry of oxidative coupling reactions.[36,37]

final products of the reaction is dependent on (1) the concentration of the diimine, (2) the nature of the oxidizing agent, (3) the pH of the reaction environment, (4) the presence of coupling agents, and (5) the presence of catalytic surfaces. The oxidation of PPDA by hydrogen peroxide to form *p*-benzoquinone diimine is relatively slow and even incomplete after 24 hours, whereas the reaction of the coupler with *p*-benzoquinone diimine is so rapid as to prevent any appreciable buildup of the quinone-imine intermediate, and to prevent completely the formation of Bandrowski's base in the dye solution (although the base may well form in the hair). The half-life of *p*-benzoquinone diimine is in the order of a few milliseconds, and its concentration under hair dyeing conditions never reaches a detectable level.[30,36,37]

Corbett[34] suggested that fading of highly colored indo dyes to light brown shades involves addition of aromatic moieties to the dinuclear indo dye. Associated with fading is hydrolytic degradation to mixtures of *p*-diamines and hydroxybenzoquinones, which, in turn, can form further colored species by their subsequent interactions. High humidity has an accelerating effect on both this type of fading and on the intramolecular cyclization of aminoindamines, which results in fading to red shades.

Analytical Methods

The Association of Official Analytical Chemists has published both a gravimetric method and an iodometric titration method for the determination of PPDA in hair dyes.[40] Colorimetric methods have been used to analyze aromatic amines, including PPDA, by their reaction with 2,6-xylenol,[41] sodium chlorite,[42] ruthenium trichloridetriphenylphosphine,[43] thiotrithiazyl chloride,[44] or peroxydisulfate,[45] or by their coupling with diazotized sulphanilic acid and other compounds.[46] A spot test for the detection of PPDA in hair dyes uses a vanillin-isopropyl alcohol reagent.[47,48] An acid-impregnated paper tape technique has also been reported.[49]

Additional methods for the separation and/or determination of PPDA or PPDA derivatives and complexes include high-pressure liquid chromatography,[50-52] gas and gas–liquid chromatography,[53-57] column chromatography on an anion-exchange resin,[58] ligand-exchange chromatography,[59] gel-permeation chromatography,[60] thin-layer chromatography,[54,61-66] thin-layer chromatography and electrophoresis,[67-71] paper chromatography,[72-74] chronopotentiometry,[75] polarography,[76,77] titrimetric techniques,[78,79] spectrophotometry,[80-82] atomic absorption spectrophotometry,[83] nuclear magnetic resonance and mass spectrometry,[84] and thermogravimetric techniques.[85]

COSMETIC USE

PPDA is used as a dye intermediate; on application to the hair with concomitant oxidation, PPDA produces a permanent color.[30,35] Data submitted to the Food and Drug Administration (FDA) in 1981 by cosmetic firms participating in the voluntary cosmetic registration program indicated that PPDA was used in a total of 500 hair coloring formulations (Table 5). Seven hair tint products on the market contained PPDA at concentrations of $\leq$0.1 percent, and 493 hair dye and

TABLE 5. Product Formulation Data[86]

Product Category*	Total No. Containing Ingredient	No. Product Formulations within Each Concentration Range (percent)		
		>1–5	>0.1–1	≤0.1
Hair dyes and colors (all types requiring caution statement and patch test)	493	57	210	226
Hair tints	7			7
1981 TOTALS	500	57	210	233

*Preset product categories and concentration ranges in accordance with federal filing regulations (21 CFR 720.4).

color formulations contained the ingredient at levels of ≤0.1 percent (226 products), >0.1 to 1 percent (210 products), and >1 to 5 percent (57 products).[86] Wall[87] reported that typical PPDA levels in color shampoos and toners may range from 0.2 to 3.75 percent and 0.05 to 0.1 percent, respectively. The highest level of PPDA in hair color products is in black shades (normally 3.5 to 4 percent).[10] Since most hair dye formulations are proprietary, exact concentrations are not available.[4]

Voluntary filing of product formulation data with FDA by cosmetic manufacturers and formulators conforms to the prescribed format of preset concentration ranges and product categories as described in Title 21, Part 720.4 of the Code of Federal Regulations (21 CFR 720.4). Because data are only submitted within the framework of preset concentration ranges, opportunity exists for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to ten-fold error in the assumed ingredient concentration.

Permanent hair dye preparations are usually packaged in two containers, one holding the intermediate mixture and the other the oxidizing agent.[35] Upon product use, the intermediate is diluted and oxidized by mixing with equal parts of the oxidant, usually 6 percent hydrogen peroxide. This process may bleach the natural hair pigment.[88] The hair dye base usually consists of an ammonium oleate soap with small amounts of detergent. Free ammonia is present to promote the oxidative color reaction and to give an "on-head" pH of approximately 9.5.[30] Other materials may be present in the dye preparation; these include reducing agents to control the rate of reaction and various ingredients to aid in penetration, sequestering, foaming, and adhesion.[89]

In permanent hair dyes containing PPDA, the reactive ingredients of the formulation penetrate the cortex of the hair where the colored compounds are formed. Color development is complete in 15 to 30 minutes. The dyeing is permanent; the oxidative dye formed with PPDA is fixed in the hair cortex and is not removed by shampooing.[35] Subsequent dyeing is necessitated primarily by the need to color new hair growth rather than by the fading of the previously colored hair. However, some off-shade fading eventually does occur, as evidenced by the

development of a red tinge. This fading is attributed to slow chemical changes in the indo dyes.[30,90]

Permanent hair coloring formulations containing PPDA are applied to or may come in contact with hair, skin (particularly at the scalp), eyes, and nails. Individuals dyeing their hair may use such formulations once every few weeks, whereas hairdressers may come in contact with products containing PPDA several times a day. Forty percent of women in the US are estimated to be regular users of hair dyes.[30] Under normal use conditions, skin contact with the hair dye is restricted to 30 minutes with a solution containing less than 3 percent PPDA.[30,36,37] Users are exposed to unreacted PPDA and couplers, as well as to reactive intermediates, particularly quinone-imine and the various indo dyes.[36,37] However, exposure to quinone-imine and the brown dye, Bandrowski's base, may be limited. Whereas the oxidation of PPDA by hydrogen peroxide to form *p*-benzoquinone diimine is relatively slow and even incomplete after 24 hours, the reaction of the various couplers with the diimine is so rapid as to prevent any appreciable buildup of the quinone-imine intermediate and to prevent completely the formation of Bandrowski's base in the dye solution (although it may form in the hair). The half-life of *p*-benzoquinone diimine is in the order of a few milliseconds, and its concentration under use conditions "never reaches a detectable level."[30,36,37]

Most permanent hair dyes on the market contain coal tar hair dyes. These dyes are no longer produced from coal but come from petrolatum. Although the term "coal tar" is archaic, it is still used in legal documents.[91,92] Coal tar hair dyes are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal Food, Drug and Cosmetic Act of 1938, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The following caution statement should be displayed conspicuously on the labels of coal tar hair dyes:

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

Patch test instructions call for a 24-hour patch with the intermediates and hydrogen peroxide mixed in the same manner as in use.[30] The irritation test is to be performed prior to each and every application of the dye. In actual practice, many beauty parlors do a 24-hour patch test prior to the initial hair dyeing procedure but omit the test on subsequent applications.[93]

NONCOSMETIC USE

In addition to its cosmetic use as a dye intermediate in permanent hair coloring formulations, PPDA is used as a photographic developing agent, a laboratory reagent, 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. The compound 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 polyparaphenylene terephthalamide, a fiber used in tire cords.[3,4,6,7,13,19,20,33,94-98]

In dye manufacturing, PPDA is used as an intermediate in the production of a number of colors having commercial significance.[4,24,99,100] These include C.I. Direct Orange 27, Disperse Yellow 9, Solvent Orange 53, Sulphur Brown 23, Leuco Sulphur Brown 23, C.I. Disperse Black 2, C.I. Direct Green 28, C.I. Acid Violet 3, C.I. Direct Black 9, C.I. Direct Black 2, C.I. Direct Black 80, and C.I. Direct Black 19. The compound is also an intermediate in the production of dyes derived from 4-aminoacetanilide, 4-aminoformanilide, 4-nitro-aniline, and 4-aminooxanilic acid.[4,24]

Chemical and biochemical applications of PPDA include use as an indicator and reagent for nitrogen,[96] as a chromogenic spray reagent for thin-layer chromatography,[101] and as a hydrogen donor for peroxidase assay systems.[102-106] PPDA is also used for removing nitrogen and sulfur oxides from waste gases[107-111] and for the colorimetric determination of hydrogen sulfide in air,[112,113] thiocyanate in biological fluids,[114] and inorganic phosphorus in serum.[115] Other applications include use as a substrate to measure the activity of oxidative enzymes[116-127] and as a staining agent for biological materials.[128-143]

The hydrochloride salt of PPDA 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. It is also used in the manufacture of rubber and plastics.[4,7,99] Derivatives of PPDA are important antioxidants in synthetic and natural rubbers, petroleum products, cellulose ethers, and alfalfa meal.[4,9]

GENERAL BIOLOGY

Numerous studies of the biological effects of PPDA have been published. PPDA added to ATP-free maintenance media at concentrations of 10 to 50 μg/ml stimulated respiratory syncytial viral growth in Hep-2 cultures.[144] Inhibition of growth occurred in *Xanthomonas oryzae* and *Xanthomonas citi* following exposure to 0.1 M PPDA,[145] and concentrations of 1000 ppm PPDA completely inhibited spore germination in *Clathridium corticola*.[146] PPDA-hydrochloride demonstrated schistosomicidal activity when given orally to mice infected with *Schistosoma mansoni*.[147] The hair dye intermediate also possessed insecticidal and tuberculostatic properties.[147,148]

In studies with mice, a "total dosage" of 67.6 mg/kg PPDA was given by intramuscular injection during either a 10- or 20-day period. The activity of various enzymes was measured 48 hours following the last injection. When compared to control animals, mice given PPDA for 10 days had a 33 percent increase in hepatic catalase activity, a 32 to 36 percent decrease in hepatic succinic dehydrogenase activity, and a 23 to 32 percent decrease in hepatic cytochrome oxidase activity; no changes were noted in blood catalase activity or in the blood peroxidase index. In animals exposed to PPDA for 20 days, a 38 percent increase in hepatic catalase activity was observed as compared to control values; no changes were noted in the hepatic activities of succinic dehydrogenase or cytochrome oxidase, in the activity of blood catalase, or in the blood peroxidase index.[149]

Inhibition of catalase activity was observed in beef liver exposed in vitro to

10^{-7}M (approximately 20 percent inhibition) to 10^{-2}M (approximately 90 percent inhibition) PPDA. Studies with the meta, ortho, and para isomers of phenylenediamine indicated inhibition of catalase activity increases in vitro, with increasing instability of the compound toward oxidation. No inhibition of hepatic catalase activity was noted in mice given injections of the para isomer (dose unspecified). The lack of action in vivo may be due to "rapid degradation" of PPDA in the organism.[150]

A test suspension of PPDA in propylene glycol was administered by intraperitoneal injection to male rats in a dose of 100 μmol/kg (in a volume of 2 ml). Blood activities of aspartate aminotransferase and alanine aminotransferase remained "essentially unchanged" from control values.[151]

Appiani et al.[152] reported that the narcotic effect of pentobarbital in rats was potentiated by pretreatment of the animals with PPDA. Microsomes from rats pretreated with PPDA also metabolized both evipan and strychnine in vitro at lower rates than did microsomes from control animals. According to the researchers, the increased drug sensitivity of individuals exposed to PPDA may have been due to a partial inhibition of hepatic microsomal enzymes.

PPDA and other trypsin inhibitors were given to starved rats by gastric intubation to determine their effect on release of pancreatic enzymes. Secretory stimulation of the pancreas by 0.01 M PPDA was not significantly different than that of the saline control. The author concluded that there was "no strict parallelism" between pancreas-stimulating activity and trypsin-inhibitory strength.[153]

Administration of PPDA-hydrochloride in 0.9 percent sodium chloride as a slow intravenous perfusion (10 mg/100 g or 20 mg/100 g) or as a rapid intravenous injection (5 mg/100 g) induced an irreversible cardiovascular collapse in rats.[154-156] The cardiovascular collapse was accompanied by a significant increase in blood catechol amine concentrations,[154] but the cause of the collapse could not be explained by a liberation of endogenous amines from mastocytes or by consumption of kininogens.[155] PPDA-hydrochloride (20 mg/100 g) given by intravenous perfusion had no effect on the elevation of blood pressure induced in rats by adrenalin or noradrenalin[154,156] and had no direct medullary excitatory action.[156]

Skeletal muscle lesions were induced in rats (weighing approximately 150 to 200 g) by means of a single, subcutaneous injection of PPDA-hydrochloride (3 mg) in aqueous solution (unspecified concentration).[157-159] Lesions occurred 1 to 24 hours after injection and were characterized by both necrosis and edema. In the diaphragm, lesions were accompanied by various myo- and neuromyopathies.[158] Serum concentrations of creatine phosphokinase and lactic dehydrogenase remained unchanged following the single subcutaneous injection.[157] Two subcutaneous injections of the PPDA solution separated by an interval of 12 hours caused an increase in the serum concentration of creatine phosphokinase, but no significant change in the concentration of lactic dehydrogenase was observed. Zones of lysed myocardial cells were also observed 24 hours after the two doses.[157] In other studies, necrotic lesions and edema occurred at the site of injection following subcutaneous administration of 1, 2, or 5 percent PPDA-hydrochloride in 0.9 percent sodium chloride. The edema was attributed to an increase in vascular permeability and was accompanied by release from tissues of histamine and 5-hydroxytryptamine.[160] Edema and focal necrosis have been observed in rats following skin application of 1 to 5 mg PPDA.[156]

Rabbits given PPDA in oral doses of 20 mg/kg per day for 12 to 13 days had increased blood concentrations of alpha-, beta-, and gamma-globulin and decreased serum concentrations of albumin and total protein. A decreased albumin:globulin (A:G) ratio was also observed. PPDA administered to rabbits daily in oral doses of 10 mg/kg for 90 days 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. The authors suggested that changes in the serum protein concentration may be related to alterations in vascular permeability.[161]

Rabbits that received PPDA at doses of 20 mg/kg for 12 to 13 days and 10 mg/kg for 90 days had marked alterations in myocardial parenchyma. These changes included edema, swelling of muscular fibers, cytoplasmic homogenization, and disappearance of cross-striation.[162]

Some aromatic amino compounds are effective inducers of methemoglobinemia,[33,163] and "humans are particularly sensitive" to compounds that induce this condition.[33] A suspension of PPDA in propylene glycol was given by intraperitoneal injection to male rats at a dose of 100 μmol/kg (in a volume of 2 ml). The percentage of methemoglobin formed in the blood was 12.9 ± 4.2 5 hours after the injection. Methemoglobin formation was also studied in vitro by incubating 0.1 μmol of rat hemoglobin with 0.5 μmol of PPDA at 37°C for 5 hours. Methemoglobin formation in vitro was 12.8 ± 0.4 percent, whereas the control methemoglobin concentration was 4.2 ± 1.0 percent.[151]

In another study investigating methemoglobin formation by PPDA, 3.23×10^{-4} mol/kg of the hair dye intermediate was suspended in 0.5 ml of peanut oil and injected intraperitoneally into male rats. Methemoglobin as a percentage of total hemoglobin was 3.7 ± 1.0 at 1 hour, 1.4 ± 0.6 at 4 hours, 3.8 ± 1.4 at 7 hours, and 3.6 ± 1.5 at 10 hours after injection. In vitro determinations of methemoblogin were also made. Rat erythrocytes were isolated and incubated with 10^{-3}M PPDA dissolved in dimethyl sulfoxide. Methemoglobin as a percent of total hemoglobin was 2.0 ± 1.8 at 1 minute, 1.2 ± 0.5 at 5 minutes, 1.8 ± 0.1 at 10 minutes, 1.8 ± 0.1 at 20 minutes, 2.4 ± 0.7 at 30 minutes, 0.5 ± 0.5 at 60 minutes, 3.9 ± 0.9 at 90 minutes, and 3.9 ± 0.9 at 120 minutes of incubation. No methemoglobin formation was observed in erythrocytes incubated with the dimethyl sulfoxide vehicle. Additional studies demonstrated that *p*-aminophenol induced methemoglobin formation. When incubated together with *p*-aminophenol in isolated rat erythrocytes, PPDA had a strong inhibitory effect on methemoglobin formation.[164]

Three groups of 2 female beagles each were bled 2 days before dosing and 6 and 24 hours after gastric intubation of aqueous PPDA solutions in doses of 1.0, 3.0, and 10.0 mg/kg. Methemoglobin concentrations in the blood were measured. In an additional trial of the same experiment, 2 more female beagles received 10 mg/kg PPDA orally. All methemoglobin values were within the normal range.[165]

A group of 10 pregnant rats received 40 mg/kg PPDA orally on Days 8, 9, and 10 of gestation. Two rats died after the third dose. A second group of 10 pregnant rats received 30 mg/kg PPDA orally on Days 6 through 15 of gestation. There were 20 control rats. The animals were bled 4 to 5 hours after the final dose of PPDA, and methemoglobin concentrations were measured as a percent of total hemoglobin. All the control animals had methemoglobin concentrations of less

than 0.1 percent. 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 percent, respectively. All other treated rats had methemoglobin concentrations of less than 0.1 percent.[166]

Massive peribronchial infiltrates of eosinophils were observed in guinea pigs 72 hours after intrapulmonary injection of an aqueous solution containing 1 percent PPDA. Antigen-induced infiltrates of eosinophils were limited to the injected lung; no eosinophilia developed in the blood, and no infiltrates of eosinophils were detected in the noninjected lung, which served as the control. The author noted that although anaphylactic sensitivity to PPDA is known, the hair dye intermediate generally induces a delayed sensitivity that is not associated with eosinophilia.[167]

Using histochemical staining techniques, Shelley and Juhlin[168] discovered a selective uptake of PPDA by Langerhans cells in isolated guinea pig and human epidermis. It was postulated that Langerhans cells were the site of hapten binding and antigen formation as well as the central target cells in immune contact dermatitis reactions involving PPDA.

Several researchers have proposed that the oxidation products of PPDA were involved in the production of allergic sensitization reactions. It has been suggested that quinonoid intermediates, such as quinonediamine, may link with skin proteins to form antigens.[35,169,170] Hapten-amino acid adducts may be the primary sensitizers in allergic contact sensitization, although the visible allergic reaction would require hapten-protein conjugation.[171] There was, however, no direct experimental evidence that the allergic behavior of *p*-phenylenediamines required the binding of quinonoid oxidation products to epidermal proteins. Evidence presented by Reynolds et al.[172] suggested that *p*-phenylenediamines differed from many contact allergens; they did not form stable isolatable adducts with epidermal amino acids. In their studies on guinea pig skin epidermis in vivo, binding between highly reactive PPDA oxidation products and skin protein was demonstrated; however, binding was relatively labile and a hapten-amino acid adduct could not be isolated. Therefore, "if the antigen in *p*-phenylenediamine hypersensitivity is an epidermal protein conjugate, it is formed by a much less stable linkage than has hitherto been thought necessary."[172]

Lerner and Fitzpatrick[173] observed that PPDA inhibits melanin formation in vitro. The hair dye intermediate combined with ortho-quinones, thereby preventing the oxidation of dopa-quinone to melanin.[173,174] Brotherton[174] found that incubation of 10 mM PPDA with cultures of both white and black pig skin 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. Brotherton[174] suggested that PPDA was an inhibitor of tyrosinase, an enzyme that catalyzes the oxidation of tyrosine to dopa and the oxidation of dopa into melanin. Inhibition by PPDA of melanin formation in vivo has not been reported.[173]

A concentration of 10^{-3}M PPDA stimulated the hexose monophosphate glycolytic pathway in isolated guinea pig brain tissue and inhibited glycolysis by the Embden-Meyerhof-Parnas pathway. Addition of the hair dye intermediate to the cerebral cortex slices as an electron acceptor caused an increase in $^{14}CO_2$ from [1-^{14}C]glucose when compared with that from [6-^{14}C]glucose.[175] Cilento and Zinner[176] suggested that in studies of electron transport in which *p*-phenylene-

diamines are used as mediators between the respiratory chain and the substrate, the substrate oxidation may, in part, bypass the respiration-phosphorylation chain. Thus, a lowering of the P:O ratio would be expected as a result of increasing concentrations of PPDA.

Studies by Loew et al.[16] suggested that the mutagenic activities of amino-substituted anilines, such as PPDA, were correlated with various "electronic parameters" reflecting potential for N-hydroxylation and stability of aryl nitrenium ions (RNH^+). Aromatic amines are known substrates of cytochrome P-450 and can undergo N-hydroxylation as well as ring epoxidations and hydroxylations. Transfer of an electrophilic oxygen atom to the substrate from cytochrome P-450 occurs in all these reactions. The ultimate form of aromatic amine that interacts with target macromolecules is postulated to be an aryl nitrenium ion. Whereas phenols formed by ring hydroxylation are usually metabolic deactiviation products, ring epoxidation is a step in the metabolic activation of polycyclic aromatic hydrocarbons to mutagens. PPDA may have a relatively high potential for epoxidation.

Clayson and Garner[15] observed that N-hydroxylation was a prerequisite for carcinogenicity of aromatic amines. Evidence reviewed by these authors suggests that "aromatic amine carcinogenesis is the result of bioactivation to the ultimate carcinogenic form and then dissociation of the reactive species to give a positively charged ion" (Fig. 4). The resulting electrophilic metabolite may subsequently react covalently with nucleophilic sites on critical macromolecules, such as DNA.[17]

Endogenous prostaglandin biosynthesis in homogenates of rat brain was inhibited 58 to 67 percent by 2×10^{-4}M PPDA.[177]

No hepatic toxicity was observed in male rats given a single intraperitoneal injection of PPDA in propylene glycol at a dose of 100 μmol/kg (in a volume of 2 ml).[151]

Glutathione depletion, lipid peroxidation and cell lysis were observed in isolated rat hepatocytes treated with 1.0 mM PPDA.[178]

PPDA added to rat peritoneal mast cell cultures at concentrations of 20 to 300 ng/ml had no effect on degranulation.[156]

A 0.9 percent sodium chloride solution containing 100 μg/ml PPDA failed to induce release of histamine or 5-hydroxytryptamine when incorporated into isolated rat mastocytes.[179]

Interference with mitosis was observed in intestinal cells of mice given a 0.05 mg intraperitoneal injection of PPDA.[180,181]

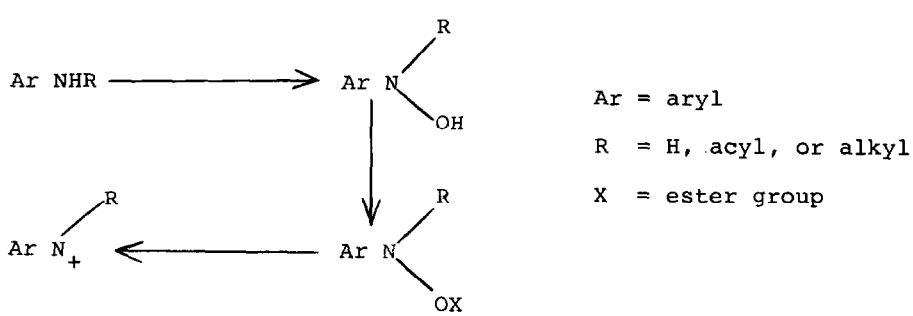


FIG. 4. Scheme for metabolic activation of aromatic amines.[15]

Cultures of the skin and of the heart muscle of chicks "ceased to develop" when exposed to 2.5 mM PPDA.[181,182]

Numerous studies were conducted during the late 1800s and early 1900s to determine the biological effects of PPDA.[13,183-187] For a summary of the biological literature on PPDA during the late 1800s and early 1900s, the reader is referred to reviews by Hanzlik[184] and von Oettingen.[188]

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Approxmately 15 μg of PPDA hydrochloride (^{3}H) diluted in normal saline was administered intravenously to a rabbit. Blood was drawn over an 8-hour period, clearance of the blood was fitted to a biexponential curve, and the curve indicated a biphasic clearance of the radioactivity from the blood with half-life values of 24 minutes and 43.5 hours. In another experiment, approximately 30 μg was applied to a shaved area on the abdomen of a rabbit. After 20 minutes, only 0.05 percent of the applied radioactivity could be detected in the blood.[189]

The tissue distribution of radioactivity was studied after intravenous and percutaneous administration of labeled PPDA to mice. PPDA hydrochloride (^{3}H) was diluted with saline and approximately 3 μg was administered intravenously. Mice were killed over a 12-day period, and tissue (liver, heart, kidney, stomach, intestine, adrenals, testes, muscle, bone marrow, bone, brain, skin, thyroid, eyes, spleen, and lungs) and blood samples were taken. Greater than 85 percent of the radioactivity was cleared from the blood within the first day. No target organ was apparent. The maximum concentration of radioactivity was located in the stomach and kidney at 1 hour, in the skin at 1 day, in the spleen at 2 days, in the muscle at 3 days, and in the bone marrow at 5 days. On the seventh day, less than 0.5 percent of the injected radioactivity was retained in any of the tissues examined.[189]

Approximately 3 μg of PPDA hydrochloride (^{3}H) was applied to two shaved areas on mice, and the applications were repeated 20 minutes later. Mice were killed over a 3-day period, and tissue (same tissues as after intravenous administration) and blood samples were taken. The percutaneous absorption of radioactivity was rapid. Despite its rapid clearance from the blood (concluded from the previous experiment), there was a steady rise in concentration of radioactivity in blood over the first 24 hours. The maximum concentrations of radioactivity at 3.5 hours were found in the brain, at 24 hours in the brain, liver, and stomach, and on the second day in the liver, stomach, and adrenals.[189]

PPDA dihydrochloride was applied in gels and fluids, such as those used in human hair dyeing, to the skin of dogs, and absorption was calculated from the concentrations of PPDA observed in the blood or the amounts excreted in the urine. Correction factors were determined by observing concentrations in blood and urine after intravenous infusion or subcutaneous injection of known amounts of PPDA dihydrochloride. Male dogs were trained to lie on their backs for 3 hours with their legs held loosely after application of PPDA dihydrochloride preparations to their abdominal skin. After the 3 hours had elapsed, the dogs were washed with soap and water and were rinsed. In the first series of experi-

ments, a gel containing 2.5 g of PPDA dihydrochloride (1.5 g PPDA) in a mixture of 25 ml of oleic acid, isopropanol, ammonia, higher alcohol sulfates (mainly lauryl sulfate), sodium ethylenediamine tetraacetate, sodium sulfite and perfume, and 25 ml of water or 6 percent hydrogen peroxide, adjusted to pH 9.5, was used. The gel with water was applied to the skin and covered with aluminum foil or left uncovered and spread from time to time with a spatula. The gel with hydrogen peroxide was applied and left uncovered. PPDA was measured in the blood after application of the gel with water, and it was determined that absorption was favored in the covered application. The blood concentrations at 3 hours were 0.15 and 0.5 μg/ml for the uncovered and covered gel with water applications, respectively. The gel was washed off the skin after 3 hours, and the PPDA concentrations in the blood slowly dropped over the next 3 hours. Absorption was stimulated by a continuous intravenous infusion of PPDA dihydrochloride into anesthetized dogs. The amounts of PPDA that must have been absorbed to result in various blood concentrations of PPDA were calculated. The intravenous infusion with constant velocity did not perfectly simulate the absorption of PPDA through the skin. It was calculated that 11 mg/kg of PPDA was absorbed when the gel with water was applied under cover to the skin. In the gel with water application without cover, a total of 16 mg of PPDA was absorbed. No PPDA was found in the blood when gel with H_2O_2 was used for skin application. Since intravenous infusions of 0.001 mg/kg per minute of PPDA yielded detectable blood concentrations, the absorption must have been less than 2 mg. In a second series of experiments, a fluid containing 0.6 percent PPDA in 30 ml of detergent, a phosphoric acid ester of a higher alcohol, sodium hydroxide, and perfume, 10 ml of isopropanol, and 10 ml of water, adjusted to pH 9.5, was applied to the skin of dogs. PPDA absorption was calculated from urinary excretion. The absorption was estimated by determining the amount excreted after the subcutaneous injection of known quantities. Generally, PPDA measured in the urine was proportional to the subcutaneous dose. Even though the concentration of PPDA in the fluid was lower than in the gel, the total amount of PPDA absorbed was approximately the same from the fluid as from the uncovered gel with water application. Absorption was less if resorcinol and 2,4-diaminophenol were added to the fluid preparation. Bandrowski's base, an oxidation trimer of PPDA, was absorbed through the skin and was detected in the urine, although not in the blood.[190]

PPDA (^{14}C) as the free base was applied in a dose of 4 μg/cm² to the forearm of 6 human subjects. Within 5 days 12.7 ± 6.98 percent of the radioactivity was recovered in the urine. PPDA (^{14}C) was applied as the dihydrochloride to the same subjects, and 14.8 ± 5.5 percent of the radioactivity was recovered in the urine. Metabolic transformation was not evaluated in this study. Additionally, these values would be expected to be higher if they were corrected by adjusting for amounts found in urine after intravenous injection of known amounts of PPDA.[88]

PPDA (^{14}C) was added to a commercially available hair dye, and the manufacturer's instructions were used to dye the hair of rhesus monkeys and humans. Three monkeys were anesthetized, and the dye lotion (2.5 g of the dye solution and 2.5 g of 6 percent hydrogen peroxide) was worked into their dry scalp hair for approximately 3 minutes and left on for an additional 20 minutes, and then the hair was rinsed, towel-dried, and shaved. Urine was collected from the mon-

keys at 6, 12, and 24 hours, and then at 24-hour intervals for 7 days. The same dye mixture (approximately 110 g) was applied to the dry hair of 5 humans. It was worked in for 5 to 8 minutes and left on for an additional 20 minutes. The hair was rinsed, towel-dried and shaved. The subjects collected urine for the time periods 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours, and then at 24-hour time periods up to 144 hours. Radioactivity determinations were made on the hair and urine. The 3 monkeys excreted in their urine ranges of 0.083 to 0.190 percent and the 5 humans excreted in their urine ranges of 0.072 to 0.207 percent of the applied radioactivity during the 7 days following hair dye use. Ranges of 12.9 to 14.5 percent of the applied radioactivity were measured in the hair of the monkeys, and ranges of 14.1 to 26.5 percent of the applied radioactivity were measured in the hair of humans.[191] Hair dyes are normally used once every 6 weeks, and maximum exposure to PPDA would occur in users of black oxidation hair dyes. These users would apply approximately 100 g of a dye composition containing up to 2 g of PPDA on each occasion. If approximately 0.2 percent of the applied PPDA was absorbed percutaneously, a 50 kg person would absorb 80 μg/kg at each hair dye application.[10]

PPDA has a low octanol/water coefficient; therefore, there may be little potential for bioaccumulation. However, no data exist either to support or disprove this. PPDA has the potential to be converted, either metabolically or chemically, to compounds, such as quinones, hydroxylated or acetylated derivatives, and azo and azoxy derivatives, that may be of toxicological significance for humans and other organisms.[13]

PPDA (^{14}C) was administered intraperitoneally in doses of 1.5 mg/kg to male rats, and the radioactivity was followed over time. The highest concentration of radioactivity was found 1 hour after administration in tissues other than the intestines, and this was followed by a rapid decrease in concentration. Large amounts of radioactivity were found in the intestinal tract and in the urine. Only very small amounts of radioactivity were found in the tissues after 48 hours. Sixty-nine percent of the administered radioactivity was excreted in the urine, 29 percent was excreted in the feces, and 26 percent was in the bile within 24 hours. The male rats rapidly excreted PPDA after its intraperitoneal administration. N,N'-diacetyl-PPDA, *p*-aminoacetanilide, and unchanged PPDA were identified as urinary metabolites. Thirty percent of the radioactivity in the urine was accounted for by N,N'-diacetyl-PPDA.[192]

PPDA-hydrochloride (^{3}H) was administered to rabbits by subconjunctival injection, intravitreal injection, local drops, and subcutaneous injection into the head. The aqueous fluid from the anterior chamber of the eye was examined for radioactivity. There was rapid clearance from the site of administration. Detectable amounts of radioactivity were found in the aqueous fluid 15 to 30 minutes after administration. The peak concentration was reached within a half-hour for subconjunctival injection and local drops and within 1 hour for subcutaneous and intravitreal injection. With the exception of application by subcutaneous injection, the concentration of radioactivity in the aqueous chamber fluid was less than 5 percent of the peak concentration after 4 days. Thirty percent of the peak concentration of radioactivity in the aqueous chamber fluid was found 4 days after subcutaneous injection. The half-life of clearance of PPDA from the aqueous chamber fluid after subcutaneous injection was 3.8 $\pm$ 0.5 days.[193]

ANIMAL TOXICOLOGY

Oral Studies

Acute Toxicity

The acute oral toxicities of PPDA and hair dye formulations containing PPDA have been studied in rats,[194-196] rabbits, cats,[184] and dogs[165] (Table 6). The animals received the material by single-dose gavage and were observed for 14 days. The LD_{50} value for rats was 80 mg/kg in one study[194] and 98 mg/kg in another study.[196] In the Hodge and Sterner[197] classification of single-dose oral toxicity for rats, PPDA would be classified as moderately toxic.

Subchronic and Chronic Toxicity

Subchronic and chronic oral toxicity of PPDA dihydrochloride has been studied in rats and in mice by the National Cancer Institute.[99] PPDA dihydrochloride was fed to rats and mice at dietary concentrations ranging from 68 to 3160 ppm and 100 to 4640 ppm, respectively, for 7 weeks, and the animals were observed for another week. There were 10 animals at each of 11 doses and there were 10 controls. All the animals survived and no signs of toxicity were observed. The compound was administered in the feed at concentrations of 625 and 1250 ppm to groups of 100 rats and groups of 100 mice for 103 weeks. The rats were observed for 2 weeks and the mice for 1 week following the feeding experiment. Forty animals of each species were controls. No body weight depression or other signs of toxicity were observed in the treated animals. (See Carcinogenesis Section for further information on these experiments.)

An aqueous solution of PPDA was administered orally in a dose of 40 mg/kg to 10 pregnant rats on Days 8, 9, and 10 of gestation and in a dose of 30 mg/kg to 10 pregnant rats on Days 6 through 15 of gestation. There were 20 control rats. A decrease in the body weight of the rats given 40 mg/kg PPDA was observed on Days 9 and 10. Two rats given 40 mg/kg died after the third dose. No other differences were observed between control and treated rats.[166]

Dermal Studies

Acute Toxicity

The acute dermal toxicity of PPDA and PPDA-containing products to rabbits has been studied. The dry, basic form of PPDA and a 10 percent alcoholic solution of PPDA applied to an approximately 25 cm^2 area of shaved, washed, and dried skin of 3 rabbits resulted in no demonstrable signs of systemic toxicity.[184]

A hair dye composite formulation containing 1.2 percent PPDA was applied in a dose of 10 g/kg to the skin on the backs of 2 male and 2 female rabbits. The hair on the backs of the rabbits had been clipped, and the application sites were approximately 10 percent of the body surface. After application of the composite, the exposure site was wrapped with impervious plastic sheeting for 24 hours. The animals were observed for 14 days following the treatment. The percutaneous LD_{50} of the formulation was greater than 10 g/kg.[198]

TABLE 6. Acute Oral Toxicity of PPDA

Material Tested	*Doses of PPDA*	*No. and Species of Animals*	*LD_{50}*	*Comments*	*Reference*
PPDA in oil-in-water emulsion	–	10 rats at each dose	80 mg/kg	–	194
PPDA as base and hydrochloride in water	0.1–0.45 g/kg	7 rabbits	–	Minimum fatal dose was 0.170 g/kg. Increase in pulse and respiration and decrease in temperature. Facial, tongue, and neck edemas and dysphea observed in 4 rabbits receiving from 0.2 to 0.22 g/kg. These 4 rabbits eventually died, although there was recovery from the edema and other symptoms. High-dose rabbits died before edema developed	184
PPDA as base and hydrochloride in water	0.1 g/kg	1 cat	–	Increase in pulse and respiration and decrease in temperature	184
PPDA in water containing 0.05 percent Na_2SO_3 and adjusted to pH 7.0	–	2–10 rats at each dose	98 mg/kg	–	196
PPDA in water	1.0, 3.0, and 10.0 mg/kg	2 female beagles at each dose	–	The 10.0 mg/kg dose dogs showed lacrimation at 2 hours and redness and swelling of conjunctiva at 3 to 6 hours. The eyes were slightly cleared at 24 hours. At 3 hours, the 3.0 mg/kg dose dogs showed slight redness and lacrimation and the 1.0 mg/kg dose dogs showed lacrimation. The lower-dose groups were normal at 24 hours	165
PPDA in water	10.0 mg/kg	2 female beagles	–	At 2 to 6 hours, the dogs developed red and swollen conjunctiva and ocular discharge. At 24 hours the dogs had half-closed eyes and red and swollen conjunctiva	165
Hair dye composite containing 1.156 percent PPDA	–	2 male and 2 female rats at each dose	41.3 ml/kg for male rats and 38.3 ml/kg for female rats	–	195

Subchronic and Chronic Toxicity

Long-term dermal toxicities of PPDA and hair dye formulations containing PPDA have been studied in mice,[199-202] in rabbits,[202-204] and in rats[205] (Table 7). PPDA, in a concentration of 10 percent in acetone and applied dermally in a dose of 0.02 ml twice a week to mice for their lifetime or to rabbits for 85 weeks, was not toxic. Hair dye composite formulations containing up to 4 percent PPDA were mixed 1:1 with hydrogen peroxide and were applied topically 2 times a week in a dose of 1 mg/kg for 13 weeks to rabbits or once a week in a dose of 0.025 ml for 2 years to mice or twice a week in a dose of 0.5 ml for 2 years to F_{1A} rats (and to the F_0 parents from the time of their weaning to the weaning of the F_{1A}); no toxic signs were observed.

Primary Skin Irritation

PPDA and hair dye formulations containing PPDA have been tested for primary skin irritation in a variety of animal species: these include rabbits,[184,196,198,206-209] guinea pigs, mice, miniature pigs, piglets, dogs and baboons[207] (Table 8). In one experiment with an aqueous slurry of 50 percent PPDA, the primary irritation index (PII) (scale = 0 to 8) for mice was 0.8, for guinea pigs was 0.5, for rabbits was 2.0, for miniature pigs was 0.4, for piglets was 1.8, for dogs was 0.5, and for baboons was 0. In other experiments the PII of PPDA for rabbits varied from 0.3 (a 2.5 percent aqueous solution) to 3.4 (0.05 g/ml in olive oil). The PII for rabbits of a hair dye containing 1.8 percent PPDA was 1.25.

Skin Sensitization

Experiments have been conducted with PPDA and with hair coloring formulations containing PPDA[208-217] (Table 9). PPDA was a strong sensitizer in guinea pigs using a variety of test methods; induction routines and challenge patches with 0.001 to 10 percent PPDA sensitized 56 to 100 percent of guinea pigs on test. A hair-coloring formulation containing 2 percent PPDA did not sensitize any of 12 guinea pigs. The results of Herve-Bazin et al.[208] indicated that 80 percent of treated guinea pigs were sensitized to 0.05 percent PPDA in petrolatum. Cross-reactions were also observed: 95 to 100 percent of the treated guinea pigs were also sensitized to 0.5 percent in petrolatum of each of the amine antiozonants, N-phenyl-N'-cyclohexyparaphenylenediamine (CPPD), N-dimethyl-1,3-butyl-N'-phenylpara-phenylenediamine, and N-isopropyl-N'-phenylpara-phenylenediamine (IPPD). Results of a study with PPDA in the guinea pig lymphocyte transformation test correlated well with sensitivity.[218] Maguire[219] reported that a hair dye with 28 percent PPDA derivatives was a strong guinea pig skin sensitizer.

Sensitization may be transferred from a sensitized guinea pig to a nonsensitized guinea pig by the transfer of intact, sonicated, or disrupted cells,[220] by the transfer of lymph nodal or splenic cells,[221] or by arteriovenous cross-transfusion or parabiosis.[222]

Eye Irritation

The rabbit ocular irritation of PPDA and products containing PPDA has been investigated in several studies. Lloyd et al.[196] placed 0.1 ml of a 2.5 percent aqueous PPDA solution containing 0.05 percent Na_2SO_3 and adjusted to pH 7.0

TABLE 7. Subchronic and Chronic Dermal Toxicity of PPDA

Material Tested	*Concentration (percent) of PPDA*	*Method*	*Length of Study*	*No. and Species of Animals*	*Results*	*Reference*
PPDA in acetone	5, 10	Application of 0.02 ml PPDA 2 times a week to a 1 cm diameter regularly shaved area of interscapular skin	Lifetime	Female mice; 50 per dose and 100 controls	Average lifespan unaffected. Normal behavior and no significant changes in body weight or food intake. No treatment-related ulceration or dermatitis was observed	202
PPDA in acetone	5, 10	Application of 0.02 ml PPDA 2 times a week to the inside left ear	85 weeks	Female rabbits; 5 per dose and 5 controls	No observed differences in food intake, weight, behavior, or overall appearance. Survival rate unrelated to treatment. No definite signs of toxicity. No differences in blood and urine parameters. No local skin changes	202
4 hair dye composite formulations	1.0, 2.0, 3.0, or 4.0 in the hair dyes	Mixed 1:1 with H_2O_2. 1 mg/kg applied 2 times a week to 2 clipped, alternated, sites on the back. Sites on ½ of the animals were abraded once a week. Rabbits were restrained for 1 hour following dye application and then were shampooed, rinsed and dried	13 weeks	Groups of 12 rabbits, 3 control groups	No evidence of compound-induced toxicity. Body weight gain was normal. Blood and urine parameters and organ weights were not significantly different from controls. No gross abnormalities observed at necropsy. No microscopic lesions due to hair dye administration	203
3 hair dye experimental formulations	1.50 in the hair dyes	Mixed 1:1 with H_2O_2. 0.05 ml applied weekly or fortnightly to shaved mid-scapular skin	18 months	Groups of 100 mice, 250 control mice	No overt signs of systemic toxicity. Survival time and body weights comparable. Liver weights in the range of normal values. All blood parameters within normal limits. Microscopic examination showed skin and appendages normal. Moderate alopecia in about half of the mice receiving the hair dyes weekly	199

2 hair dye formulations	1.50	Mixed 1:1 with H_2O_2. 0.05 ml applied weekly to clipped intrascapular region and dried with a hair dryer without heat	2 years	28 male and 28 female mice in each group, 76 male and 17 female mice in control group	No skin irritation observed. No significant differences in body weight gains. Survival rate of all mice was erratic	201
Hair dye composite formulation	1.2	Repeated treatment group received 1 g/kg daily 5 times a week for 65 applications on shaved abdomen. The skin of 4 rabbits in this group and control group was abraded. The as used group received 10 g/application massaged into their back fur 10 minutes, allowed to remain an additional 20 minutes, then rinsed and dried, once every 2 weeks for 7 exposures	13–14 weeks	5 male and 5 female rabbits in treated and control groups	Accumulation of dye on shaved animals resulted in fissures, scab formation, and desquamation; no observations on unshaved group. Mild to marked acanthosis and mild to moderate dermal fibroplasia in exposed skin of 7 rabbits in repeated test group. No differences between intact and abraded skin. Moderate acanthosis and very mild dermal fibroplasia in exposed skin of 2 animals in as used group. Hematological analyses, clinical blood chemistry, and urinalyses normal. Gross pathology revealed equivocal kidney lesions in test groups, was not confirmed histopathologically	204
4 hair dye composite formulations	1.0, 2.0, 3.0, or 4.0 in the hair dyes	Mixed 1:1 with H_2O_2. 0.025 ml applied once/week to a clipped intrascapular region. 20 mice from each group killed and necropsied at 7 and at 9 months	21–23 months	Groups of 50 male and 50 female mice, 3 control groups	No differences observed in mean absolute and relative liver and kidney weights, and survival rates	200
3 hair dye composite formulations	2.0, 3.0, or 4.0 in the hair dyes	Mixed 1:1 with H_2O_2. Applied topically to F_0 generation from	2 years	60 male and 60 female rats per group, 3 con-	Dry skin noted in first few weeks of study in 15–20 percent of female rats and slightly decreased mean values for total erythrocytes,	205

TABLE 7. (Continued)

Material Tested	*Concentration (percent) of PPDA*	*Method*	*Length of Study*	*No. and Species of Animals*	*Results*	*Reference*
		time of their weaning to the weaning of F_{1A}. F_{1A} received 0.2 ml of the hair dye increased by 0.1 ml weekly to 0.5 ml, 2 times a week on the clipped neck and back. 10 rats from each group killed and necropsied at 12 months		trol groups (from F_{1A} generation)	hemoglobin, and hematocrit observed in male rats receiving hair dye containing 3 percent PPDA. No other differences observed in general behavior, appearance, biochemistry, and urinalyses	

TABLE 8. Primary Skin Irritation by PPDA

Concentration (percent) of PPDA and Vehicle	*Method*	*No. and Species of Animal*	*Results*	*Reference*
50 percent, aqueous slurry	1 24-hour occluded patch application to clipped, intact and abraded skin. Erythema and edema reactions are evaluated (0–4) at 24 and 72 hours, and values are added and averaged to yield a primary irritation index (PII scale = 0–8). Dog and baboon PII are estimates; no abraded sites	6 each of mice, guinea pigs, rabbits, miniature pigs, piglets, dogs and baboons	PIIs for mice, guinea pigs, rabbits, miniature pigs, piglets, dogs, and baboons were 0.8, 0.5, 2.0, 0.4, 1.8, 0.5, and 0, respectively. Rabbit and piglet showed flare-up skin reactions 2 to 3 weeks after application	207
Dry, basic compound	Shaved, washed, and dried a 25 cm^2 area of skin. Rubbed PPDA into it	3 rabbits	No effects observed	184
10 percent alcoholic solution	Shaved, washed, and dried a 25 cm^2 area of skin. Applied PPDA	3 rabbits	Mild erythema observed	184
0.5 g/ml, water; 2.5 percent, petrolatum; 25 percent, petrolatum; 0.05 g/ml, olive oil	0.5 ml of the water or olive oil or 0.5 of the petrolatum applied to intact and abraded skin. Erythema and edema reactions are evaluated (0–4) at 24 and 72 hours, and values are added and averaged to yield a PII (scale = 0–8)	6 rabbits per treatment	PII = 3.2 PII = 1.4 PII = 2.3 PII = 3.4	208
2.5 percent, aqueous solution containing 0.5 percent Na_2SO_3 and adjusted to pH 7.0	1 24-hour occluded patch application to clipped intact and abraded skin. Erythema and edema reactions are evaluated (0–4) at 24 and 72 hours and values are added and averaged to yield a PII (scale = 0–8)	3 rabbits	Very slight edema at the abraded sites of 2 rabbits. In 1, the reaction ameliorated in 72 hours. PII = 0.3	196
5 percent, ethanol	1 24-hour occluded patch application to clipped skin. The skin of half the animals was abraded. Erythema and edema reactions are evaluated (0–4) at 24 and 72 hours and values are added and averaged to yield a PII (scale = 0–8)	8 rabbits	PII = 0.88	209
Hair dye, 1.2 percent	Approximately 10 percent of the body surface clipped (the backs). Applied 10 g/kg, wrapped in impervious plastic sheeting for 24 hours	2 male and 2 female rabbits	Slight to moderate erythema and moderate edema observed	198
Hair dye, 1.8 percent	Applied 0.1 ml daily for 3 days to intact shaved skin. Graded at 24 hours. PII (scale = 0–8) determined on day greatest irritation observed	9 rabbits	PII = 1.25. Mildly irritating	206

TABLE 9. Skin Sensitization

Material Tested	*Concentration (percent) of PPDA*	*Method*	*No. of Guinea Pigs*	*Results*	*Reference*
PPDA in 70% ethanol	2	24-hr occluded patch of 0.5 ml on back read 1,7,24, and 48 hours later. Induction, intradermal injection of 0.1 ml Freund's complete adjuvant diluted to 50% in saline on Days 0 and 9, occluded patch of 0.5 ml on Days 0, 2,4,7,9,11,16,18, and 21 to clipped back. Tenth patch removed Day 23. Challenge, Day 35, 48-hour occluded patch of 0.5 ml to another back site. Reactions read at 0, 7,24, and 48 hours, erythema scored scale of 0 to 4. Histological examination, challenge sites with macroscopic reactions	20	20/20 reacted. Mean erythema score was 2.16. 2/3 had allergic type inflammatory reactions with intense spongiosis and massive lymphocyte exocytosis. One showed necrosis with erosion, weeping, and a squamous crust	210
PPDA in olive oil for injection induction, in petrolatum for dermal induction and challenge	Injection induction with 0.5, dermal induction with 1, challenge with 0.05 and 0.5	Induction, intradermal injection of PPDA and complete Freund's adjuvant. 1 week later, cutaneous application. Challenge 1 week later by cutaneous application. Control animals challenged only	20 per treatment	0.05 and 0.5% of PPDA sensitized 80 and 100% of the animals, respectively. 0.5% PPDA irritating to 20% of the nonsensitized controls	208
PPDA in petrolatum	Induction with 2, challenge with 0.1, 0.5, 1, and 2	Induction, 4 24-hour occlusive patches of 0.5 g on alternate days on clipped flank. After 14 days, challenge on opposite flank in occlusive chamber. Reactions scored 24, 48, and 72 hours, erythema scored scale of 0–3	10 at each of 4 challenge concentrations	0.5, 1, and 2% sensitized all animals. Mean erythema scores were 2.2, 2.1, and 1.5, respectively. 0.1% sensitized 4/10. Mean score was 0.5	213
PPDA in propylene glycol for intradermal induction, in 70% ethanol for topical induction, and in 95% ethanol for challenge	Intradermal induction with 0.005, topical induction with 0.05 or 0.1 for larger animals, challenge with 0.5, 1, and 5	Guinea pig maximization test[214] (scale = 0–3)	2 groups of 25 females	14/25 and 16/25 positive reactions. Mean scores were 0.65 and 0.71, respectively	214

PPDA (99%) in physiological saline for induction and intradermal challenge, in petrolatum for epidermal challenge	Intradermal induction and challenge with 0.1, epidermal challenge with 1	Induction, 3 intradermal injections of 0.1 ml in a week and 3 intradermal injections of 0.1 ml of 1:1 Freund's adjuvant:saline with PPDA 2 weeks. After 14 days, challenge, intradermal injection of 0.1 ml into fresh flank site and 24-hour occluded patch 14 days later at a different site. Challenge reactions read 24-hours after injection or patch removal	20	20/20 and 13/20 positive sensitization reactions after intradermal and epidermal challenges, respectively	215
PPDA in water	Intradermal induction with 0.1, topical induction with 5, challenge with 1	Guinea pig maximization test[214] (scale = 0–3)	10	All animals were sensitized. Mean erythema + edema score was 2.9	209
PPDA in water	Primary irritation with 0.001, 0.01, 0.1. Induction and challenge with 0.001	Primary irritant effect determined after intradermal injection. Induction, intradermal injection over 10 days with 0.1 ml. 17 days later, challenge, intradermal injection of 0.1 ml. Reactions read 48 hours later, biopsies were performed	20 on test, 18 controls	No primary irritation reactions. 16/20 positive sensitization reactions. High correlation between microscopic and macroscopic reactions	216
PPDA in dimethyl formamide	Induction with 10, challenge with 1	Induction, 0.1 ml applied to outer ears for 3 days; 4 days later, challenge, 0.2 ml applied to depilated flank. Erythema scored 24 hours later. Controls were challenged noninduced animals	8	8/8 positive (pink) reactions	217
PPDA	Induction injection with 2.5, challenge injection with 1.0, challenge application with 5.0	4 simultaneous induction injections at sites overlaying axillary and inguinal lymph nodes. Challenge by intradermal injection and open topical application on opposite shaved flanks 14 days later. Reactions graded at 24 hours	10	All the guinea pigs were sensitized; a strong sensitizer	212
PPDA	Intradermal induction with 0.25, topical induction with 0.5, challenge with 0.5	Guinea pig maximization test[214] (scale = 0–3)	10	90% of the guinea pigs were sensitized; a strong sensitizer. Mean of all positive challenge scores was 2.45	212

TABLE 9. (Continued)

Material Tested	*Concentration (percent) of PPDA*	*Method*	*No. of Guinea Pigs*	*Results*	*Reference*
PPDA	Induction injection of 0.25, challenge patch of 1.0	Single intradermal injection of PPDA and Freund's complete adjuvant in nuchal region. Challenged 12–14 days later with 6 hour occluded chamber application on a shaved flank. Reactions graded 18 and 42 hours later (scale = 0–3)	10	All the guinea pigs were sensitized; a strong sensitizer. Mean of all positive challenge scores was 2.1	212
Hair coloring formulation	2	Material diluted to 1% in propylene glycol. 9 0.1 ml topical inductions on the shaved back over 3 weeks. Challenge 2 weeks later at the original and an untreated site. Observed at 24 and 48 hours	12 female	No positive reactions were observed; not a contact sensitizer	211

in one eye of each of 3 rabbits. Ten seconds later the eyes were irrigated with 50 ml of lukewarm water. The researchers observed mild conjunctival inflammation that did not persist for more than 24 hours.

Morikawa et al.[209] used the method and scoring system of Draize et al. to determine the irritation of 100 percent PPDA in rabbit eyes. The maximum irritation score reported was 17.0 of a possible 110.

A hair dye composite formulation containing 1.2 percent PPDA was tested for ocular irritation with 10 rabbits. One-tenth milliliter of the dye was instilled into the conjunctival sac of one eye of each animal. The hair dye was rinsed from the eyes of 5 animals with 40 ml water 4 seconds after instillation. The maximum possible irritation score was 110. For the unwashed eyes, the average irritation score at 1 hour was 33.0, at 24 hours was 34.0, at 48 hours was 24.0, at 72 hours was 14.0, at 96 hours was 9.0, and at 7 days after instillation was 2.4. For the washed eyes at the same times after instillation the scores were 23.0, 20.0, 10.0, 7.0, 4.0, and 0, respectively.[223] A similar study was conducted with a hair dye containing 1.8 percent PPDA. One-tenth milliliter was instilled into the conjunctival sac of one eye of 6 rabbits. The maximum possible irritation score was 110. The average scores were 30 for 1 day, 29 for 2 days, 19 for 3 days, 15 for 4 days, and 6 for 7 days after instillation. The hair dye was moderately irritating to the eyes of rabbits.[224]

Other Studies

The rat acute intraperitoneal LD_{50} of an aqueous PPDA solution was 37 mg/kg.[194] The intraperitoneal administration of 190 mg/kg of PPDA hydrochloride to rats and 120 mg/kg to cats resulted in edema of the head and neck.[186]

Hanzlik[184] reported that the minimum fatal dose of PPDA base and hydrochloride in water administered subcutaneously to 10 rats and 3 rabbits was 170 mg/kg and 200 mg/kg, respectively. Edema of the head and neck was observed in some of the rabbits. In another study, the subcutaneous administration of 350 mg of PPDA hydrochloride to guinea pigs and doses of 120 to 150 mg to rats resulted in edema of the head and neck.[187]

The minimum fatal dose of PPDA base and hydrochloride administered intravenously to 3 dogs was 170 mg/kg.[184]

SPECIAL STUDIES

Animal Reproduction and Teratology

PPDA in aqueous solution was administered by gavage in doses of 5, 10, 15, 20, and 30 mg/kg per day on Days 6 through 15 of gestation to groups of 25 pregnant rats. A control group given only water and a pair-fed control group (paired to 30 mg/kg per day group) were also included in the experiment. The rats were killed on Day 20. Feed consumption was significantly reduced during the dosing period for the 20 and 30 mg/kg per day groups. There were significant decreases in feed consumption for several days during the dosing period for the 15 mg/kg per day group. Rats in the 20 and 30 mg/kg per day groups and the pair-fed controls had significantly reduced weight gains over the dosing period, but these results were not significant when compared to the controls over the 20 days. Two

pregnant rats in the 30 mg/kg per day group died. There were no other maternal deaths. No significant differences in the numbers of corpora lutea, implantation sites, live fetuses or resorptions, or in fetal weights and male:female ratios were observed. There were no biologically meaningful or statistically significant increases in the numbers of litters or fetuses with soft tissue or skeletal malformations in any of the treatment groups.[225]

An aqueous PPDA solution was administered subcutaneously in a dose of 28 mg/kg to three groups of 25 mice on Days 5 to 7, 8 to 10, or 11 to 14 of gestation. There were 25 control mice. Small increases over the controls in average resorption numbers and percent fetal skeletal variations were observed in the treated groups; the researchers claimed that these increases were within the range of values found for historical controls. They concluded that there was no evidence PPDA had an embryotoxic or teratogenic effect.[226]

A 0.2 percent aqueous solution of PPDA was administered intraperitoneally to groups of 20 male rats three times a week for 8 weeks at doses of 2, 6, and 20 mg/kg. Forty control rats were injected with sterile water. After the 8 weeks, each male was mated weekly with 2 females for 4 weeks. Seventeen days after separation from the males, the females were killed, their uteri were examined, and the number of dead and live fetuses and implantation and absorption sites were counted. No evidence of an increase in postimplantation fetal loss was observed.[194,227]

Hair dye products containing 1.0, 2.0, 3.0, or 4.0 percent PPDA were applied topically in doses of 2 ml/kg to groups of 20 pregnant rats on Days 1, 4, 7, 10, 13, 16, and 19 of gestation. The rats were shaved 1 day prior to each application, and the dyes were mixed with an equal volume of 6 percent hydrogen peroxide just before their use. There were three negative control groups. The rats were killed on Day 20 of gestation. No biologically significant soft tissue or skeletal changes were observed in the embryos. The mean numbers of corpora lutea, implantation sites, live fetuses, resorptions per pregnancy, or litters with resorptions were not significantly affected by the dye treatments. The litter sex ratios and pregnant rat weight changes and feed consumption were similar for untreated controls and dye-treated groups.[203]

A hair dye formulation containing 3 percent PPDA was mixed 1:1 with 6 percent hydrogen peroxide and applied to the clipped backs of 50 female mice two times a week for 4 weeks prior to mating and through the mating and gestation periods. There was evidence of mating in 34 treated mice, and 26 became pregnant. Each mouse received 0.05 ml of the dye and hydrogen peroxide mixture at each application. There was evidence of mating in 30 control mice, and 23 became pregnant. No overt signs of maternal toxicity were observed. One treated animal died prior to gestation; it had a discolored liver and an enlarged spleen. The maternal weight gains and pregnancy and mortality rates of the treated mice were comparable to the controls. The mean numbers of implantations, live fetuses, and resorptions and fetal sex ratios, and numbers of skeletal and soft tissue malformations were similar in treated and control mice. Slightly lower fetal weights were observed in the treated mice, but the mean crown–rump distances were comparable to the controls. The researchers concluded that there was no evidence of a teratogenic effect. However, there may have been a retarding ef-

fect on the ossification process, particularly of the bones of the feet and of the cervical and caudal vertebral centra.[228]

The same hair dye formulation (3 percent PPDA) was mixed 1:1 with 6 percent hydrogen peroxide and applied to the clipped backs of 34 female rabbits two times a week for 4 weeks prior to mating and through the mating and gestation periods. All 34 rabbits were mated, 26 became pregnant, and 3 died during the gestation period. The rabbits received 4.0 ml/kg applications of the mixture of hair dye with hydrogen peroxide. Of the 34 control rabbits, 32 were mated, 21 became pregnant, and 6 died during gestation. No overt signs of maternal toxicity were observed. No adverse effects on pregnancy rates and maternal survival and body weights were found. Focal alopecia was noted at slightly higher incidences in treated rabbits during the first two thirds of gestation; in the last third of gestation, the incidence of alopecia in control and treated rabbits was similar. The mean numbers of corpora lutea, implantations, live fetuses and resorptions, implantation efficiency, and number of doses with two or more resorptions were comparable in control and treated rabbits. There was no evidence of a teratogenic effect. There may have been some evidence of embryotoxicity; the percent of live fetuses was less in the treated rabbits (85.4 percent in the treated rabbits and 93.8 percent in the control rabbits), and the fetal sex ratio (male:female ratio of 0.7) was unusually low. However, there was no adverse effect on the weight or length of the fetuses that survived to Day 30 of gestation.[229]

Hair dye formulations containing 2, 3, and 4 percent PPDA were mixed 1:1 with 6 percent hydrogen peroxide and applied two times a week to the clipped backs and necks of groups of 40 male and 40 female mice (the F_0 generation). The initial dose level was 0.2 ml of the dye per application and this was increased by 0.1 ml/application weekly to a dose of 0.5 ml/application. Treatment was continuous through growth, mating, gestation, and lactation to the weaning of the F_{1B}, F_{2B}, and F_{3C} litters of the respective generations. There were three control groups. The dye-treated groups were comparable to the control groups in general behavior and appearance, feed consumption, body weight gain, and survival. Treated rats had a few skin reactions throughout the study; these included mild scabbing, fissuring, loss of elasticity, and leathery texture. The treated F_0, F_1, and F_2 parents did not differ from the controls in fertility, gestation survival, and live birth indices. Litter size and body weights of the young were similar. No treatment-related gross or microscopic lesions were observed in the F_{1B} parental rats or F_{3B} weanling rats killed and necropsied during the study. No treatment-related gross lesions were observed in the rats that died during the study.[230]

A dye product containing 2.20 percent PPDA was applied topically to 25 male rats two times a week for 10 weeks. The dye product was applied in a dose of 0.5 ml to two alternating shaved back sites. The dye was mixed with an equal volume of 6 percent hydrogen peroxide before use. There was a control group of 25 rats. After the 10 weeks, each male rat (P_0) was mated to 1 female each week for 3 weeks. Each of 100 male offspring from these matings (F_1 males) was mated to 1 female per week for 3 weeks. These female rats were killed at between 14 and 16 days of gestation. There were no significant effects on dye-treated P_0 male body weight gains. There were no differences in P_0 male percent fertility or total and average live pups per F_1 litter. There was no indication of reduced fertility in

the F_1 males. The numbers of implantations, dead fetuses, and resorptions were similar for the treated and control groups.[231]

Mutagenesis and Carcinogenesis

Short-Term Tests

Bacterial Mutagenesis

Many studies have been conducted on PPDA using the Ames test and modifications of the Ames test (Table 10). All PPDA and PPDA and hydrogen peroxide mixtures were negative in the Ames test in the absence of metabolic activation (Table 10). Both positive and negative results were reported for PPDA and PPDA and hydrogen peroxide mixtures with metabolic activation. Crebelli et al.[232] reported that purified PPDA was negative and commercial PPDA was positive in the Ames test with metabolic activation. They suggested that the positive result may have been due to impurities in the commercial PPDA.

Other researchers have disagreed with this conclusion. Burnett et al.[247] reported fresh and aged aqueous solutions of PPDA, and fresh DMSO solutions of PPDA were nonmutagenic in the Ames test. Aged DMSO solutions of PPDA (used up to 4 hours after dilution) were mutagenic. Even after aging, PPDA in acetone or ethanol and DMSO solutions of PPDA containing 15 percent water were nonmutagenic. Nishi and Nishioka[248] determined that DMSO solutions of PPDA kept in the dark were not mutagenic in the Ames test but that the same solutions exposed to fluorescent light for 10 minutes to 4 hours were mutagenic. A sample of the light-exposed PPDA was analyzed by thin-layer chromatography, and the amount of Bandrowski's base in the sample increased with exposure time and the mutagenicity paralleled the concentration of the base; Bandrowski's base may have been responsible for the observed mutagenicity.

Five oxidation products of PPDA were tested for mutagenicity in the Ames test, and all were more mutagenic than PPDA. Bandrowski's base and *p*-nitroaniline were positive in strain TA1538 with metabolic activation; 4,4-azodianiline and *p*-dinitrobenzene were positive in strains TA1538 and TA100 with metabolic activation; and 2-(4'aminoaniline)-5-hydroxy-1,4-quinonediimine was positive in strain TA1538 without metabolic activation. A solution of PPDA oxidized with hydrogen peroxide in the presence of *m*-amines, such as 2,4-toluenediamine and 2,4-diaminoanisole, was more mutagenic than a solution of PPDA alone.[251]

An aqueous solution of PPDA was administered intraperitoneally to groups of 20 male rats in doses of 2, 6, and 20 mg/kg three times a week for 8 weeks. Their urine was tested in 10 percent DMSO in the Ames test with strain TA1538. Compared to the DMSO vehicle their urine was not mutagenic.[227] Crebelli et al.[232] performed the Ames test with strain TA98 with and without metabolic activation on urine concentrates from rats treated topically with PPDA/resorcinol conjugates. The urine concentrates induced mutations only with metabolic activation. The urine concentrates of untreated rats did not induce mutations. Fifteen women collected their urine before and after using hair dyes containing 0.46 to 2.55 percent PPDA. The urine was tested in DMSO in the Ames test in strain TA1538 with metabolic activation. The urine was not more mutagenic after hair dye application than before hair dye application.[10,252]

Nishioka[253] examined PPDA in the *Escherichia coli* DNA repair test. He found that PPDA inhibited *E. coli* growth.

Mutagenicity in Drosophila melanogaster

Blijleven[254,255] used the induction by PPDA of sex-linked recessive lethal mutations in *Drosophila melanogaster* as a measure of the mutagenicity of PPDA. In Blijleven's original study,[254] 5.1 and 15.5 mM PPDA in DMSO and sucrose solution was fed to adult males for 3 days. The treated males were individually mated to 3 females in a 3-day brood period and then were mated with two groups of 3 females in two consecutive 2-day brood periods. The first brood represented mainly treated sperm, and the second and third broods represented treated spermatids (and sperm) and treated spermatocytes (and spermatids), respectively. Mutation induction was detected as a lack of certain male progeny in the second generation after the treated generation. The results of this study indicated a weak mutagenic activity of PPDA with peak mutagenic activity in spermatids and spermatocytes. However, concern was expressed about the purity of the PPDA used in this experiment, and Blijleven[255] repeated the experiment with the same PPDA and with a new sample of PPDA (base) of higher purity dissolved in water. The original PPDA was fed to the flies at a concentration of 15.5 mM and the new PPDA at concentrations of 2.5, 5, 10, and 15.5 mM. The original PPDA sample caused a significant mutation frequency increase when compared to the control mutation frequency. When all the data were pooled and compared to the controls, no significant differences were observed in mutation induction. The same experiment was performed after the injection of 2.5, 5, or 10 mM high purity PPDA (base) into adult males. The toxicity of the compound was a problem, and there was a high degree of sterility in the treated males. When the injection data were pooled and compared to the pooled control data, the differences were not significant. Blijleven concluded that PPDA was not mutagenic to *D. melanogaster* and that the impurities in the original sample of PPDA may have accounted for the observed mutagenic effects.

Micronucleus Test in Rats and Mice

A suspension of PPDA in 0.5 percent gum tragacanth containing 0.05 percent sodium sulfite was administered orally to 10 rats in two 500 mg/kg doses 24 hours apart. Six hours later the animals were killed, the femurs were dissected out, and bone marrow smears were prepared. The smears were examined microscopically, and the number of micronucleated cells per 2000 polychromatic erythrocytes per animal was determined. These values were compared with the values obtained from rats treated only with the vehicle. No clear evidence of mutagenic potential for PPDA was found.[256] PPDA was reported to be inactive in a mouse micronucleus test.[257]

Inhibition of Mouse Testicular DNA Synthesis

Seiler[258] administered PPDA orally in a dose of 200 mg/kg to 3 or 4 male mice and then determined the amount of labeled thymidine incorporated into testicular DNA. PPDA depressed almost all testicular DNA synthesis, suggesting that it was genetically active.

TABLE 10. Ames Test: *Salmonella*/Mammalian-Microsome Mutagenicity Test

Material Tested	*Method*	*Results*	*Reference*
PPDA, PPDA and H_2O_2 mixture	Ames et al.[233]: Spot test (~ 1 mg) with and without S-9 (9000g supernatant of rat liver homogenate. Rats induced with polychlorinated biphenyl mix (Aroclor 1254)). Strains TA100, TA97, TA1538, and TA98	PPDA alone had no mutagenic activity. PPDA and H_2O_2 mixture gave a very strong mutagenic response with TA1538 when S-9 was present	234
Purified PPDA in water, 2 commercial samples of analytical PPDA in in water, PPDA and resorcinol in 50 percent NH_4OH and with H_2O_2	Ames et al.[233]: Plate incorporation with and without S-9. Strain TA98. Microtiter fluctuation test with microsomal activation, method of Gatehouse and Delow[235]	Purified PPDA (0–2 mg/plate) produced no significant increase in number of revertants. Both commercial samples of PPDA (0–2 mg/plate) and the PPDA/resorcinol/H_2O_2 mixture (0–1.0 mg/plate) increased the number of mutants in the presence of S-9. These results were confirmed in the microtiter fluctuation	232
PPDA in DMSO	Ames et al.[233], slightly modified by Nagao et al.[236]: Liver S-9 from rats induced with polychlorinated biphenyl (Kanechlor 500). Preincubation of PPDA and bacteria with and without S-9 at 37°C (for 20 minutes) followed by plate incorporation. Strains TA100 and TA98	PPDA (0.5–2 μmol/plate) was significantly mutagenic to TA98 in the presence of S-9	237
PPDA hydrochloride	Ames et al.[233]: Plate incorporation with S-9 from uninduced and induced rats and mice. Strains TA1535, TA100, TA1537, TA1538, and TA98	PPDA (0.1–6.6 mg/plate) was not mutagenic without activation but was mutagenic in TA1535, TA100, TA1538, and TA98 with induced mouse and rat liver S-9. With uninduced rat liver S-9, there was no mutagenic activity with TA1535 and reduced activity with TA1538 and TA98	238
PPDA in DMSO	Ames et al.[233]: Plate incorporation with liver S-9 from uninduced rats and mice and animals induced with B-naphtholfavone. Strain TA1538	No mutagenic activity with uninduced S-9. Slight mutagenic activity with induced rat and mouse liver S-9	239
PPDA in DMSO	Ames et al.[233], slightly modified: Plate incorporation with and without liver S-9 from rats induced with phenobarbital. Strain TA1538	PPDA (50 and 100 μg/plate) was significantly mutagenic in the presence of S-9	240
PPDA	Ames et al.[233], slightly modified by Nagao et al.[236]: Strains TA100 and TA98	PPDA was mutagenic only in TA98 in the presence of S-9	241

PPDA in water, PPDA in 2 percent NH_4OH, PPDA in 2 percent NH_4OH and with H_2O_2	Ames et al.[233]: Plate incorporation with and without S-9 from noninduced and induced rats. Strains TA1535, TA100, TA1537, TA1538, and TA98	PPDA (5–1000 μg/plate) was not mutagenic without induced rat liver S-9; PPDA was slightly mutagenic to TA1538 and TA98 with induced rat liver S-9. PPDA (250–1000 μg/plate) was not mutagenic to TA1538 and TA98 with S-9 from noninduced rat liver; slight increase in revertant colony number with PPDA and NH_4OH and no activity with PPDA, NH_4OH and H_2O_2 in TA98 in the presence of S-9 from induced rat liver	242
PPDA, PPDA and H_2O_2	Ames et al.[233]: Plate incorporation with and without S-9. Strains TA1535 and TA1538	PPDA alone was slightly mutagenic with TA1538 in the presence of S-9. PPDA and H_2O_2 mix was mutagenic with TA1538 in the presence of S-9	243
PPDA in buffer, PPDA and H_2O_2	Ames et al.[233]: With and without S-9. Strain TA98	PPDA in buffer and PPDA and H_2O_2 (15–150 μg/plate of PPDA) were bacteriostatic without S-9. PPDA in buffer and PPDA and H_2O_2 (50 and 150 μg/plate of PPDA) were mutagenic in the presence of S-9	244
PPDA in buffer, PPDA and H_2O_2	Ames et al.[233], slightly modified by Nagao et al.[236]: Plate incorporation or spot test with and without S-9. Strain TA98	PPDA in buffer and PPDA and H_2O_2 (0.003–1346.153 μg/plate of PPDA) had no mutagenic activity without S-9. PPDA in buffer and PPDA and H_2O_2 (13.461 and 134.615 μg/plate of PPDA) were mutagenic in the presence of S-9	245
PPDA in DMSO	Ames et al.[233]: Preincubation for 37°C for 1 hour with and without S-9 followed by plate incorporation. Liver S-9 was from rats, hamsters and mice induced with polychlorinated biphenyls, 3-methylcholanthrene, and phenobarbital or uninduced. Strain TA98	PPDA was not mutagenic with all hamster S-9 and with S-9 from rats and mice induced with polychlorinated biphenyls and 3-methylcholanthrene	246
PPDA in water, PPDA in DMSO	Ames et al.[233]: With S-9, fresh and aged (used 0–4 hours after dilution) DMSO solutions. Strains TA1538 and TA98	All aqueous solutions and the fresh DMSO solution of PPDA were nonmutagenic with S-9. Aged DMSO solutions were mutagenic with S-9	247
PPDA in DMSO	Ames et al.[233]: With S-9, exposed to Toshiba fluorescent lamps (15W X 2) at 10 cm for 0–4 hours. Strain TA98	PPDA solution kept in the dark was not mutagenic with S-9. All solutions illuminated 10 minutes to 4 hours were mutagenic with S-9	248
3 liquid hair dyes and 2 hair dye powders containing PPDA	Ames et al.[233]: With and without S-9	One liquid induced base-pair substitutions without S-9. All the others induced frameshift mutations with S-9	249
25 hair dye preparations containing PPDA	Ames et al.[233]: 25 dyes tested without S-9 with strains TA1535, TA100, TA1537, TA1538 and TA98, and 20 dyes tested with mouse liver S-9 with strain TA98	7 dyes were mutagenic with TA1538 and TA98 without S-9 and 13 were mutagenic; 2 suspect as mutagenic with S-9 with TA98	250

Induction of Mouse Sperm-Head Abnormalities

The mouse sperm-head abnormality test reflects the effects of chemical interference in spermatozoa differentiation. This test is useful in identifying compounds that may cause transmissible genetic damage in whole animals. Groups of 5 hybrid male mice were given five daily intraperitoneal injections of PPDA in saline. PPDA was administered to the mice at doses of 5, 10, and 20 mg/kg per day. Doses of 50 and 100 mg/kg per day were toxic. Five weeks later the mice were killed and sperm smears were made. Two hundred fifty sperm-heads were classified as of normal or abnormal morphology. PPDA was not active in this test.[259,260]

Hepatocyte Primary Culture/DNA Repair Test

The hepatocyte primary culture/DNA repair test measures DNA damage after exposure to chemicals by determining the amount of DNA synthesis in nonreplicating male rat hepatocytes. Tritiated thymidine is incorporated by the cells during repair synthesis. PPDA dihydrochloride was toxic to the hepatocytes at a concentration of 0.1 mg/ml and was negative in the DNA repair test at concentrations of 0.005, 0.01, and 0.05 mg/ml.[261]

Mouse Lymphoma Forward Mutation Assay

The National Toxicology Program[262] reported that PPDA dihydrochloride was positive in the in vitro L5178Y mouse lymphoma forward mutation assay with and without metabolic activation. This assay measures genetic damage at the thymidine kinase locus after exposure of mouse lymphoma cells to chemicals.

Survival of Rat Embryo Cells

Rauscher leukemia virus-infected rat embryo cells were treated with PPDA (1.85 to 3.2 μg PPDA/5.2 $\times$ 10^4 cells) for 72 hours, and cell survival was determined 6 days later. This assay measures the acquisition of attachment independence, which is manifested by increased cell survival rates. PPDA was positive in this test. Viable cell counts were greater after PPDA treatment than after treatment with solvent (unspecified) alone.[263]

Animal Carcinogenesis

PPDA has been tested for carcinogenicity by oral and topical administration to animals. NCI[99] administered PPDA dihydrochloride in the feed at concentrations of 625 and 1250 ppm to groups of 50 rats and mice of each sex for 103 weeks. The controls were groups of 20 animals of each species and sex. At the conclusion of the experiment, all animals were killed and necropsied. Both dosed and control rats had a variety of neoplasms, but these tumors were distributed almost equally between dosed and control rats. For each sex the tumor incidence was very low and was within the range normally encountered in aging rats. A variety of tumors, all previously reported to occur spontaneously in mice, were found in both the control and dosed mice. Some neoplasms did occur only, or in greater frequency, in the dosed groups, but none was considered compound-related. The researchers concluded that PPDA dihydrochloride was not carcinogenic to rats or to mice under the conditions of the bioassay. Griesemer and Cueto[264] applied the IARC[4] approach for evaluating evidence of carcino-

genicity to this NCI study and concluded that there was no evidence of carcinogenicity in these experiments.

In several studies reported 20 years earlier by Saruta et al.,[181] the oral administration of PPDA did not cause the production of any malignant tumors in rats. In the first experiment PPDA was administered daily for 8 months, in doses of 0.06 and 0.3 mg, to groups of 5 rats of each sex. PPDA was administered daily for 8 months to 5 rats in a dose of 10 mg, and these rats were compared to 5 controls in the second experiment. In the third experiment a 30 mg dose of PPDA was administered daily to 4 rats; 3 of these rats died before the experiment ended.

The lifetime percutaneous application of PPDA was studied in mice.[202] Five and ten percent solutions of PPDA in acetone were applied in a 0.02 ml volume two times a week to the shaved intrascapular skin of groups of 50 mice. There were 100 untreated control mice. Tumors were observed both in the controls and in the treated mice, but there was no significant increase in tumor incidence in the treated mice. The same investigators applied the PPDA solutions to the inside of the ears of groups of 5 female rabbits two times a week. Five control rabbits were included in the study. The experiment was terminated at 85 weeks, but at 80 weeks there were only 2 surviving rabbits in the control group, 4 surviving rabbits in the 5 percent PPDA-treated group, and 1 surviving rabbit in the 10 percent PPDA-treated group. No neoplasms were observed.

Three hair dye formulations containing 1.5 percent PPDA were mixed with equal volumes of 6 percent hydrogen peroxide just prior to use, and 0.05 ml of each was applied topically to the shaved midscapular skin of groups of 100 mice weekly or fortnightly for 18 months.[199] There were 250 control mice. No evidence of carcinogenic activity by the hair dyes was observed.

Two hair dye formulations containing 1.5 percent PPDA were mixed with an equal volume of 6 percent hydrogen peroxide just before use, and 0.05 ml was applied topically to the clipped intrascapular skin of groups of 28 male and 28 female mice weekly for 2 years.[201] There were 76 male and 17 female control mice. Male and female mice in all groups developed both benign and malignant neoplasms. There was no evidence for carcinogenicity by these two hair dye formulations.

Hair dye composite formulations containing 1, 2, 3, and 4 percent PPDA were mixed 1:1 with hydrogen peroxide, and 0.025 ml of the dyes was applied topically to the clipped intrascapular areas of groups of 50 male and 50 female mice once weekly for 21 to 23 months. At 7 and 9 months, 10 male and 10 female mice from each group were killed and necropsied. Gross and microscopic examinations were made on all mice that died during or were killed at the termination of the experiment. There were three control groups. The incidences of tumors in control and treated groups were similar. Carcinogenic effects were not induced by the hair dye formulations.[200]

Three hair dye composite formulations containing 2, 3, and 4 percent PPDA were applied topically to rats (the F_0 generation) from the time of their weaning to the weaning of their young (the F_{1A} generation). The hair dyes were mixed 1:1 with hydrogen peroxide and were applied topically two times a week for 2 years to the clipped backs and necks of groups of 60 male and 60 female rats of the F_{1A} generation. The rats received an initial application of 0.2 ml, and this was increased by 0.1 ml weekly to 0.5 ml. Ten rats from each group were killed and

necropsied at 12 months, and all other rats were necropsied at their deaths or at the termination of the experiment. There were three control groups. No compound-related gross lesions were observed. The stratum corneum of the skin and of the hair shafts of the treated rats was colored by the dye. The female rats treated with the composite that contained 4 percent PPDA had an increase in pituitary adenomas when compared statistically with all three control groups (adenomas/number females examined were 34/50, 36/51, and 35/50 for the three control groups and 45/51 for the treated group). However, pituitary adenomas have a high background incidence in rats, and they appeared in a nonsignificant pattern in all the other groups. Other lesions were seen in all the groups in low incidences.[205]

CLINICAL ASSESSMENT OF SAFETY

Dermal Studies

A 50 percent aqueous slurry of PPDA was applied to the skin of 6 subjects for 24 hours under occlusive conditions. Skin reaction was assessed at 24 and 72 hours for erythema (0 to 4) and edema (0 to 4). Erythema and edema values were added and averaged for the two skin readings to yield a PII. The PII for the PPDA on human skin was 0.8 (maximum possible total of 8).[207]

PPDA scored highly in predictive studies. Five 48-hour induction patches, containing 1 ml of 10 percent PPDA in petrolatum, with 24-hour rest periods between the patches, were applied to the forearms or calves of 24 "mostly black" volunteers from a prison. The challenge application was a 48-hour occluded patch, containing 0.4 ml of 0.5 percent PPDA in petrolatum, on the back. All 24 of the subjects were sensitized to PPDA in this maximization procedure.[265] In a second study, 10 48- or 72-hour induction occluded patches, containing 0.5 g of a 0.01, 0.1, or 1.0 percent solution of PPDA in petrolatum, were applied to the skin of male subjects. This was followed by a 2-week rest period. The challenge was a 72-hour patch with a nonirritant concentration. The 0.01 percent PPDA induction application was followed by a 0.01 percent challenge patch. Seven of 97, or 7.2 percent of the men, responded positively to the challenge. The 0.1 percent PPDA induction application was followed by a 1.0 percent challenge patch, and 11 of 98, or 11.2 percent of the men, responded positively. The 1.0 percent PPDA induction application was followed by a 1.0 percent challenge application. Forty-seven of 88, or 53.4 percent of the men, responded positively. There appeared to be an increase in the incidence of sensitization with higher concentrations of PPDA used in induction applications.[266] Epstein and Taylor[267] used a 2 percent aqueous solution of PPDA for induction applications and challenge patches in a maximization test[265] with 34 male volunteers from a correctional facility as subjects. Fifteen of the 34, or 44 percent of the subjects, were sensitized to PPDA.

Twenty-five panelists began a study with a dye composition containing 2 percent PPDA. Three panelists quit the study before the final challenge application. Semiocclusive patches containing 0.3 ml of the test material were placed on the arms. There was an initial 1-hour challenge patch with 100 percent of the dye followed by 9 24-hour induction patches with a 10 percent (v/v) aqueous solution of the dye over a 22-day period. The final challenge patch was a 1-hour patch

with 100 percent of the dye on both the original site and a new site. Reactions were scored on a scale of 0 to 7. At the initial challenge there were 20 negative reactions (scores of 0 to 1) and 5 reactions of slight dermatitis (scores of 2 to 3). Nineteen of the panelists had negative reactions during the induction period, and 6 had reactions of slight dermatitis. There were 8 reactions of no dermatitis, 7 reactions of slight dermatitis, and 7 reactions of significant dermatitis (4 to 7) at the final challenge patching.[268]

A repeated insult patch test was conducted with a hair dye containing 0.4 percent PPDA and 0.039 percent 4-nitro-*o*-phenylenediamine (4NOPD).[269] (4NOPD has also been reviewed by the Expert Panel.) Two hundred six subjects were enrolled in and completed the study. The dye was mixed with an equal volume of oxidizer, and each nonocclusive patch contained 0.1 ml/cm^2 of the dye and oxidizer mixture. Ten 48- to 72-hour consecutive patch applications were made on the backs of the subjects, and reactions were read after removal of each patch. These induction patches were followed by an 11-day rest period. A 48-hour nonocclusive challenge patch was applied to a previously unexposed site on the back of each subject, and the reaction was read at removal and at 15 minutes and at 24 hours later. There were 41 doubtful reactions (very mild erythema, barely exceeding that of untreated skin) during induction. There were no positive reactions at any induction or challenge reading. The researchers stated that their data provided no evidence to indicate that the hair dye and oxidizer test product caused either irritation or sensitization. A repeated insult patch test was conducted with a hair dye containing 0.49 percent PPDA and 0.027 percent 4NOPD on the same 206 subjects and following the same procedure.[270] There were no positive reactions at any induction or challenge reading. The researchers stated that their data provided no evidence that the hair dye and oxidizer test product caused either irritation or sensitization. A repeated insult patch test was conducted with a hair dye containing 0.596 percent PPDA and 0.049 percent 4NOPD on the same 206 subjects and following the same procedure.[271] There were no positive reactions at any induction or challenge reading. The researchers stated that their data provided no evidence to indicate that the hair dye and oxidizer test product caused either irritation or sensitization. A repeated insult patch test was conducted with a hair dye containing 2.144 percent PPDA on the same 206 subjects and following the same procedure.[272] There were no positive reactions at any induction or challenge reading. The researchers stated that their data provided no evidence that the hair dye and oxidizer test product caused either irritation or sensitization.

A variety of patch tests with PPDA have been performed on subjects from a variety of populations (Table 11). Many of these reports are of diagnostic patch tests performed on clinical subjects suffering from skin disease. The patch tests were performed with 1 or 2 percent PPDA in petrolatum, and 1.1 to 84.6 percent of the patients were positive for PPDA. PPDA is a sensitizer for human beings.

Patch and photopatch tests with 2 percent PPDA in petrolatum were performed on the back of a 52-year-old man. The photopatch site was irradiated for 15 minutes at a distance of 15 cm, 24 hours after application. The sites were scored at 24 and 48 hours after radiation. The light source was a black light (Toshiba FL20BLB) emitting wavelengths from 300 to 420 nm and consisting mainly of long-wave UV peaking at 360 nm. The papulovesicular reactions observed at the patch and photopatch sites were approximately equal.[273]

TABLE 11. Results of Patch Tests with PPDA

Material Tested	*Concentration (percent)*	*Method*	*No. and Population of Subjects*	*Results*	*Reference*
PPDA in petrolatum	1	Patch tests	108 patients with contact dermatitis of the feet correlated clinically with shoe contact (survey spans 4½ years and is from Italy)	24.8 percent (41) of the subjects were positive to PPDA	274
PPDA in petrolatum	2	Patch tests	540 patients, the majority with contact dermatitis. Reactions were scored on a 1+ to 4+ scale (from the NY Univ. Skin and Cancer Unit during 1968 to 1970)	13.5 percent reacted to PPDA. There were 12,24,26, and 11 patients with 1+, 2+, 3+, and 4+ reactions, respectively	94
PPDA in petrolatum	2	Patch tests	229 patients suspected of having contact dermatitis due to shoes or rubber. Reactions were scored on a 1+ to 4+ scale (from the NY Univ. Skin and Cancer Unit during 1968 to 1970)	7.0 percent reacted to PPDA. There were 1,3,12, and 0 patients with 1+, 2+, 3+, and 4+ reactions, respectively	94
PPDA in petrolatum	2	Patch tests on the hand	250 hospital patients and 250 private patients (survey spans 3 years and is from France)	6.8 percent (34) of the subjects gave positive responses to PPDA. Comparison of hospital and private patients showed little difference	275
PPDA	1	Al-test patches	281 housewives with contact dermatitis of the hands; 1000 people doing domestic work only (this includes the 281 women) (patients from 5 European clinics)	5 percent of both populations gave positive results to PPDA	276
PPDA in petrolatum	1	Al-test patches. Reactions read 48 and 96 hours after patch application	2806 patients, from contact dermatitis sections of hospitals or an occupational dermatitis center (from Spain during 1977)	9.90 percent (278) of the subjects were positive for PPDA. Of the 278, 14.02, 10.43, and 8.63 percent were masons, metallurgists, and housewives	277
PPDA in petrolatum	1	Patch tests	4,825 patients (from Europe)	4.9 percent (237) of the patients reacted positively to PPDA	278
PPDA in petrolatum	1	Al-test patches on the back. Results read at 48 and 72 hours	155 hospital patients, mainly outpatients (from Japan)	22.58 percent (35) of the subjects were positive for PPDA	279
PPDA in petrolatum	2	Japanese-made patches on the back. Results read at 48 and 72 hours	196 hospital patients, mainly outpatients (survey from Sept. 1973 to Aug. 1975; from Japan)	28.57 percent (55) of the subjects were positive for PPDA	279

PPDA in petrolatum	1	Patch tests on the back. Readings after 48 and 72 hours	53 denture-wearing patients with "burning mouth syndrome" (from Denmark)	1 positive reaction to PPDA. (erythema and infiltration with papules or vesicles)	280
PPDA in petrolatum	1	Patch tests	13 eczema patients allergic to a brown stocking dye (from Finland)	11 patients were positive for PPDA	281
PPDA in petrolatum	1	Patch tests	362 eczema patients (survey from Mar. 1 to Sept. 30, 1979; from Finland)	2.5 percent (9) of the subjects were positive for PPDA	281
PPDA in petrolatum	1	Patches applied, removed at Day 2. Reactions read at Day 2 and Day 4	225 men and 175 women with hand eczema (from Belgium)	9.3 percent (21) of the men and 14 or 8 percent of the women reacted positively to PPDA	282
PPDA in petrolatum	2	Patch tests on thigh or back	5558 patients (survey spans 1 to 2 years and is from 6 clinics in Scandinavia)	4.5 percent of the subjects reacted positively to PPDA	283
PPDA	–	Patch tests on back removed at Day 2 and read at Days 2 and 7. (Other chemicals tested simultaneously). Positives were reapplied on Day 7 and removed and read on Day 9	35 patients (from Canada)	17 percent (6) positive test reactions on Day 2 and 5 or 14 percent positive test reactions on Day 9	284
PPDA in petrolatum	2	Occluded patches applied to the back. Patches removed at 48 hours and read at 48 and 96 hours	536 patients (tested in 1976, from Brazil)	1.1 percent of the subjects reacted positively to PPDA	285
PPDA in water	1	Closed patch test for nonsensitized subjects and open patch test for sensitized subjects	32 hairdressers who had never suffered from contact allergic dermatitis due to PPDA and 7 hairdressers who had strongly positive reactions to PPDA (from Japan)	0/32 and 6/7 positive reactions for the nonsensitized and sensitized hairdressers, respectively	209
PPDA	1	Patch tests	2363 tested (1978–1979; from USA)	7 percent (157) positive reactions for PPDA	286
PPDA	1	Patch tests	2094 tested (1979–1980; from USA)	6 percent (136) positive reactions for PPDA	286
PPDA in petrolatum	1	Patch tests	184 men and 116 women suspected of having contact dermatitis (from Brussels)	13.0 percent (24) of the men and 4.3 percent (5) of the women were positive for PPDA. 9.7 percent of all the subjects were positive for PPDA	287

TABLE 11. (Continued)

Material Tested	*Concentration (percent)*	*Method*	*No. and Population of Subjects*	*Results*	*Reference*
PPDA in petrolatum	1	Al-test patches. Patches removed after 48 hours and reactions recorded at 48, 72, and/or 96 hours	1200 patients from private and outpatient clinics (tests from Jan. 1, 1971 to June 30, 1972; from North America)	8 percent (98) of the patients reacted positively to PPDA	288
PPDA in petrolatum	1	Al-test patches. Patches removed after 48 hours and reactions recorded at 48, 72, and/or 96 hours	3041 patients from private and outpatient clinics (tests from July 1, 1972 to June 30, 1974; from North America)	6.1 percent of the patients reacted positively to PPDA	289
PPDA in petrolatum	1	Patch tests	66 hairdressers with eczema (1973–1981; from Canada)	45 percent (30) were positive for PPDA	290
PPDA in petrolatum	1	Patch tests on the back for 48 hours. Read at 48 and 96 hours	200 hospital clinic patients with eczematous dermatitis (1977–1979; from Canada)	30 percent of the patients were positive for PPDA	291
PPDA in petrolatum	1	Patch test on the upper back for 48 hours. Read at 48 and/or 72 hours	149 patients from private practices and clinics with cosmetic-related contact dermatitis (1977–1980; from USA)	16 percent (24) were positive for PPDA	292

PPDA-sensitive subjects were exposed to open and closed patches of a commercially available hair dye containing PPDA and mixed with hydrogen peroxide and two experimental hair dyes containing PPDA and mixed with 5 and 15 ml of hydrogen peroxide. Two of 13 subjects with open patches and all of 12 subjects with closed patches reacted positively to the commercially available hair dye treated with hydrogen peroxide. Four of six subjects with open patches reacted positively to one experimental hair dye developed with 5 ml of hydrogen peroxide, and all of 12 subjects with closed patches and 4 of 7 subjects with open patches reacted positively to the same experimental hair dye developed with 15 ml of hydrogen peroxide. The use of a second experimental hair dye developed with 5 ml of hydrogen peroxide resulted in 4 positives out of 6 subjects with open patches and 12 positives out of 12 subjects with closed patches. The researchers suggested that, even after developing a hair dye with hydrogen peroxide, available PPDA and intermediaries are left and can produce reactions in sensitive individuals.[267]

Marzulli et al.[88] described a clinical case in which a beautician developed generalized urticaria when working with hair dyes; PPDA caused a wheal- and flare-response on the skin of his back. Vesiculation, oozing, crusting, and marked edema of the skin of the face, neck, ears, and scalp resulted after another man dyed his own hair; his eyes were swollen shut. A patch test to PPDA was positive.[293] The skin around the eyes is swollen frequently in hair dye dermatitis. Immediate hypersensitivity may sometimes be a component of contact dermatitis.[88]

Dermatitis covering the whole scalp, neck, chest, and both ears was observed in a man who had dyed his hair 1 week previously. Patch tests were positive for 2 percent PPDA in petrolatum for his own hair and for the hair dye. Incomplete oxidation might have been more likely to occur when the dye was not applied by an expert hairdresser.[294] Three case studies have been reported in which men suffered from eczema on their arms. In two studies, the men had positive patch tests to 1 percent PPDA in petrolatum and to their wives' dyed hair.[295,296] In the third study, the man's eczema flared up whenever his partner had just dyed her hair. Patch tests to 1 percent PPDA in petrolatum and to the hair dye were positive, and a patch test to his partner's hair dyed 1 week previously was negative. More recently dyed hair might have resulted in a positive reaction.[297] A study was conducted with 20 subjects who had suffered allergic contact dermatitis from PPDA and had strongly positive patch tests to PPDA. The subjects were patch tested with hair containing PPDA dyed 24 hours previously. The patches were repeated 3 weeks later. There were no reactions at the end of 48 hours or 3 weeks. The results of this study conflict with the patch test results of most of the case studies.[35,298]

Cross reactivity has been observed between PPDA and PPDA dihydrochloride. Eleven men and 11 women who were sensitive to 1 percent PPDA in petrolatum were patch tested with 1 percent PPDA dihydrochloride in petrolatum. Only 3 of the men and 6 of the women were sensitive to PPDA dihydrochloride. No subjects who were negative to PPDA were positive to PPDA dihydrochloride.[299]

Allergic sensitivity to PPDA has been associated with cross sensitization to azo and aniline dyes; to the local anesthetics, procaine and benzocaine; to *p*-aminobenzoic acid, its esters, and sunscreens containing them; to IPPD, used

in rubber tires; to CPPD; to *p*-aminosalicylic acid; to hydrodiuril; to carbutamide; to pyrogallol; to sulfonamides; to hydroquinone; to hydrochlorothiazide; to *p*-hydroxybenzoic acid esters; to benzidine; to phenylhydrazine; and to the hair dye, *p*-toluenediamine. These potential sensitizations have far-reaching implications.[94,208,209,267,293,300-303]

A hair dye company in New York City performed preliminary patch tests on approximately 3500 prospective models (total number of individual hair dye applications was 116,647) with hair dye composites containing up to the maximum amount of PPDA used in the product line (3.5 percent) over the period 1975 to 1983. (Virtually all of the dyes contained PPDA.) Two hundred five positive reactions were observed in 163 women. Most of the women who reported reactions had later applications of hair dyes. Only 8 reactions on 4 women were identified as allergic responses to the products.[304]

Other Studies

The use of PPDA-containing hair dyes on the hair has been accompanied by edema of the eyelids and conjunctiva and tearing. Infrequently, there is limitation of eye movements, loss of the corneal epithelium, and cellular infiltration of the stroma. The cornea usually recovers rapidly. More severe reactions occurred after the application of PPDA-containing hair dyes to the eyebrows and eyelashes. Generally, rapid onset of pain and burning of the eyes was accompanied by redness and swelling of the lids and edema and hyperemia of the conjunctiva. In some persons, the corneal epithelium was eroded and accompanied by iritis and iridocyclitis. Vision has occasionally been lost or permanently damaged by severe corneal ulceration.[31] A woman who had dyed her eyebrows and eyelashes with a product containing PPDA developed conjunctivitis within 3 days of application and then developed corneal ulcers in both eyes. Treatment included removal of eyelashes and eyebrows. Two and one-half months following product application, the woman was "feeling better," and six and one-half months following application she was able to see "fairly well."[305]

The threshold limit value (TLV) for PPDA set by the American Conference of Governmental Industrial Hygienists[19,306] is 0.1 mg/m^3 and agrees with the recommended British industry standard. This value is considered low enough to minimize the number of people who become sensitized but not to prevent asthma in humans already sensitive to PPDA. NIOSH reported that 25 mg/m^3 of PPDA is the concentration immediately dangerous to life or health.[22]

EPIDEMIOLOGY

A number of published studies assess whether occupational exposure to and use of hair dyes increases the risk of cancer. These studies do not distinguish which of the specific hair dye ingredients were involved in the human exposure. There is some controversy over whether occupational exposure to hair dyes increases the risk for bladder cancer,[307-310] and lung cancer[311,312] or use of hair dyes increases the risk for bladder cancer in men or women[313] and breast cancer in women[314-319] (Table 12).

Clemmesen[320] discussed the difficulties implicit in epidemiological studies

and reviewed many of the papers that investigated the relationship of the risk of cancer to occupational exposure to or use of hair dyes. According to Clemmesen most researchers used samples too small to allow conclusions, and analyses of duration and intensity of exposure, lag time, and the influence of lifestyle factors such as tobacco were deficient in many cases. Clemmesen believes there was no evidence of any carcinogenic effect from hair dyes on the organs investigated among the occupations and users examined.

SUMMARY

PPDA is an aromatic amine that has been used in permanent hair dyes for over 100 years. It is mixed with hydrogen peroxide immediately before use, and the resulting oxidation products react with sulfhydryl groups present in hair to form permanent bonds. Data submitted to FDA in 1981 indicated that PPDA was used in a total of 500 hair-coloring products at concentrations up to 5 percent.

Coal tar hair dye products, including those containing PPDA, are exempt from the principal adulteration provision and the color additive provisions in sections 601 and 706 of the Federal Food, Drug and Cosmetic Act of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The following caution statement should be displayed conspicuously on the label of coal tar hair dyes:

> 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.

PPDA has a variety of biological effects; it is absorbed and excreted by both animals and humans. Radioactivity was found in the blood of rabbits after the intravenous and dermal administration of radioactive PPDA (^{14}C). Radioactivity was distributed throughout the body and in the blood after the intravenous and topical administration of PPDA to mice. In dogs PPDA was found in the blood after its topical and intravenous administration and was excreted in the urine after its topical and subcutaneous administration. PPDA (^{14}C) was applied topically to humans and radioactivity was found in the urine. When a hair dye containing PPDA (^{14}C) was used on monkeys and by humans, radioactivity was detected in the hair and in the urine. PPDA (^{14}C) 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.

PPDA has a low octanol/water coefficient and, thus, may have little potential for bioaccumulation. However, PPDA may be converted, either biologically or chemically, to compounds of toxicological concern. Labeled PPDA was administered intraperitoneally, to rats and radioactivity was distributed throughout the body and excreted in the urine, feces, and bile. N,N'-diacetyl-PPDA, *p*-aminoacetanilide, and unchanged PPDA were identified as urinary metabolites.

The acute oral LD_{50} of PPDA for rats ranged from 80 to 98 mg/kg; PPDA is classified as moderately toxic. Dietary PPDA at concentrations of 3160 ppm to rats and 4640 ppm to mice for 7 weeks and 1250 ppm to rats and mice for 103

TABLE 12. A Summary of Reports on Cancers Associated with Exposure to Hair Dyes

Population Studied	*Comments*	*Reference*
	Occupational Exposure to Hair Dyes	
1030 bladder papilloma and carcinoma patients were interviewed for occupational history in Leeds, England, from 1959 to 1967. 383 male and 57 female bladder tumor patients were matched for sex, age decade, habitat, and smoking habits with 340 male and 50 female surgical controls and 312 male and 39 female patients with cancer at other sites	There were consistently nonsignificant differences found for male hairdressers (predominant occupation). There were 4, 1, and 0 hairdressers among 383 bladder tumor patients, 340 surgical controls, and 312 cancer controls, respectively. Men employed as hairdressers for less than 20 years were less likely to have bladder tumors than those employed for longer than 20 years; male hairdressers with bladder tumors had lower mean ages at diagnosis compared to the whole interviewed series. The population of males with bladder tumors contained more hairdressers than expected; 5 were observed, 1.8 and 1.5 were expected in 1961 and 1951, respectively (based on census data)	307
461 persons of ages 20–89 with transitional or squamous-cell carcinoma of the lower urinary tract (94 percent had a bladder tumor) interviewed for occupational history in an 18-month period in an area of eastern MA. 356 male and 105 female persons with a bladder cancer were matched for sex, age, and/or smoking with 374 male and 111 female controls	Cigarette smoking was not responsible for an indirect association of bladder cancer risk and occupation. Of the persons with bladder cancer, 4 were male barbers and 1 was a female hairdresser. 7.2 and 0.9 were expected, respectively. The researchers stated that the data do not support a suggestion of increased bladder risk for barbers, but that the number of observations was too low and therefore, inadequate to exclude the possibility of increased risk. No excess risk was found for female hairdressers	308
702 patients with presumptive or confirmed diagnosis of bladder tumors. 493 bladder cancer patients (265 male whites, 69 male blacks, 112 female whites, and 47 female blacks) and 527 patient controls were interviewed for occupational history from 1958 to 1964 in New Orleans, LA.	There was no clear correlation between bladder cancer and occupation or industry. For male whites with bladder cancer, 4 were barbers at the time or had been barbers as a final occupation. 1.45 were expected. The researchers had doubts about the validity of their analytical method and did not conclude that being a barber increased risk to bladder cancer. Further interviews with 7 male barbers and 2 female hairdressers with bladder cancer indicated wide differences in their occupation, starting ages, years in occupation, and age at diagnosis of bladder cancer	309
300 male and 70 female bladder cancer patients from 1957 to 1961 in New York City were matched by sex and age with the same number of control patients. All the subjects were interviewed about their occupations	There were 4 hairdressers in the male bladder cancer group, 3 of whom had been hairdressers for more than 5 years. There were no hairdressers in the male control group. There was one beautician in the female bladder cancer group. The researchers drew no definite conclusions	310

The death certificates of 3460 adult (≥ 14 years of age) females who died of cancer and 1000 females who died from some other cause in Alameda County, CA from 1958 to 1962 were examined. Cancer cases and controls were matched for age and sex	24 of the 3460 females who died of cancer and 4 of the 1000 controls were beauticians. The risk of cancer death for beauticians was elevated but not significantly. Six of the 24 beauticians who died of cancer and 170 of the 3436 females of other occupations who died of cancer had lung cancer. The small numbers inject uncertainty, but the researchers suggested that the risk of lung cancer may be substantially increased among beauticians	311
Examined hospital records from Los Angeles County for 1972 to 1975. 22792 white women with cancer, 20 to 64 years old, were admitted and 9524 of the women reported occupations	Of the 22792 women, 135 were beauticians, and 20 of the beauticians had lung cancer. 32, 22, and 15 percent of the beauticians had breast, genital, and lung cancer, respectively. Only the lung cancer incidence was significant compared to the expected frequencies for age and sex calculated from the census data	312
	Use of Hair Dyes	
107 bladder cancer patients and 107 controls were matched by age (± 5 years) and sex. Male controls were patients with benign prostatic hypertrophy, female controls had been seen with problems of stress incontinence (Toronto, Ontario, Canada)	No statistically significant difference was found between cancer and control groups in reported exposure to hair dyes	313
Surveyed 120,557 married, female, registered US nurses, from 10 states. 38,459 (31.9 percent) had used permanent hair dyes and 3548 (2.9 percent) had had cancer	Statistically significant associations with hair dye use were found only for cancers of the cervix uteri and vagina and vulva. Women who had used hair dye ≥ 21 years prior to diagnosis of breast cancer had significantly greater risk for all sites—mostly due to the excess number of observed to expected cases of breast cancer. However, those who used hair dyes 16 to 20 years prior to diagnosis of breast cancer had an almost equal deficit of observed to expected breast cancers. Adjustments for smoking did not change the results. The researchers concluded that there was no evidence of increased risk of cancer during the initial 20 years	314
191 female breast cancer patients matched for age (within 3 years), marital status, and social class with 561 inpatient, outpatient, or general practice controls (Oxford, England, 1975–1976)	There were no significant differences in the use of hair dyes by breast cancer patients and controls. The frequency of applications and brands used by hair dye users in both groups were approximately the same. There were no significant differences when the analysis was restricted to women who had used hair dyes >4 or >9 years prior to breast cancer diagnosis.	315

TABLE 12. (Continued)

Population Studied	*Comments*	*Reference*
118 breast cancer patients of ages 20 to 84 (from 3 upstate New York counties). 233 controls selected by "random digit dialing" of the telephone. Cancer patients and controls matched by age and county	No significant differences observed between breast cancer patients and controls in exposure to hair dyes. Hair dye use was marginally significantly associated with breast cancer in women 40–49 years old. Previous benign breast disease and hair dye exposure significantly increased a woman's risk of developing breast cancer. A significant dose-response relationship between number of hair dye exposures and breast cancer was observed for women who did not have gray hair and used hair dyes to change their natural hair color	316
Reviewed case histories of 100 breast cancer patients. Compared these to a study of women of the same age who did not have breast cancer (New York)	87/100 of the breast cancer patients were regular users of permanent hair coloring and had used hair dyes for more than 5 years. 26 percent of the women without breast cancer were regular users of permanent hair dyes over prolonged periods	317
129 breast cancer patients and 193 female controls without breast cancer selected from a breast cancer screening center in New York City from 1964 to 1976	There were no significant differences between the cancer patients and controls in use of hair dyes prior to breast cancer diagnosis. However, there was a difference in the integral (frequency × duration) use of dyes for the 2 groups. The association between integral use and breast cancer was clearest when hair dye was used for at least 10 years prior to cancer diagnosis. The association of integral use and breast cancer occurred primarily among women 50 to 79 years old	318
401 breast cancer patients and 625 age-matched controls without breast cancer from a cancer referral center in New York City from 1979 to 1981	There were no significant differences between the breast cancer patients and controls with regard to hair dye use: frequency, duration, type, shade, or application time. Important confounders of hair dye use included religion and smoking status	319

weeks did not result in any signs of toxicity. Oral administration of 40 mg/kg PPDA to pregnant rats resulted in the deaths of 2 of 10 rats and decreased body weight. No other signs of toxicity were observed at the 40 mg/kg dose or at a 30 mg/kg dose.

No signs of toxicity were observed when dry PPDA or a 10 percent alcoholic solution of PPDA was applied to a 25 cm^2 area of the skin of rabbits. The percutaneous LD_{50} for rabbits of a hair dye composite containing 1.2 percent PPDA was greater than 10 g/kg. Chronic topical administration of 10 percent PPDA solutions was not toxic to mice and rabbits. Subchronic and chronic dermal administration of hair dye products containing up to 4 percent PPDA was not toxic to mice, rabbits, and rats.

Primary skin irritation by 2.5 to 100 percent PPDA varied from none to slight in experiments with rabbits, guinea pigs, mice, miniature piglets, piglets, dogs, and baboons. A hair dye containing 1.2 percent PPDA produced slight to moderate erythema and moderate edema in the skin of rabbits. Another hair dye containing 1.8 percent PPDA was mildly irritating to the skin of rabbits. PPDA is a guinea pig sensitizer at induction concentrations as low as 0.001 percent. A hair-coloring formulation containing 2 percent PPDA was not a guinea pig sensitizer.

Mild conjunctival inflammation that did not persist for more than 24 hours was observed after the instillation of a 2.5 percent aqueous PPDA solution into rabbit eyes. The maximum irritation score was 17.0 out of a possible 110 after 100 percent PPDA was placed in rabbit eyes. A hair dye composite formulation containing 1.2 percent PPDA and one containing 1.8 percent PPDA were instilled into the conjunctival sacs of the eyes of rabbits. The former was rinsed from the eyes of half of the animals 4 seconds after instillation. The average irritation scores of the composite containing 1.2 percent PPDA at 1 day postinstillation was 33.0 for unwashed eyes and 23.0 for washed eyes. At 7 days postinstillation, the average irritation scores were 2.4 for unwashed eyes and 0.0 for washed eyes. The average irritation score of the hair dye containing 1.8 percent PPDA at 1 day postinstillation was 30 and at 7 days postinstillation was 6.

The acute intraperitoneal LD_{50} of an aqueous PPDA solution for rats was 37 mg/kg. The subcutaneous minimum lethal doses of PPDA were 170 mg/kg for rats, 200 mg/kg for rabbits, and 100 mg/kg for dogs. Intraperitoneal administration of PPDA to rats and cats and subcutaneous administration of PPDA to rats, rabbits, and guinea pigs resulted in edema of the head and neck.

Doses of 5 to 30 mg/kg per day of PPDA by gavage to pregnant rats did not affect reproduction, and PPDA was not teratogenic. Subcutaneous administration of 28 mg/kg per day PPDA to pregnant mice did not result in embryotoxic or teratogenic effects. No evidence of an increase in postimplantation fetal loss occurred when male rats received 2 to 20 mg/kg PPDA intraperitoneally three times a week for 8 weeks and then were mated.

Hair dyes containing 1.0 to 4.0 percent PPDA were applied to the skin of pregnant rats at a dose of 2 ml/kg per day after being mixed with an equal volume of hydrogen peroxide. No adverse effects on reproduction were observed, and the hair dyes were not teratogenic. A hair dye containing 3 percent PPDA was mixed with hydrogen peroxide, and 0.05 ml of the mixture was applied dermally two times a 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 percent PPDA 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. The percent of live fetuses was 85.4 percent in the treated rabbits and 93.8 percent 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 percent PPDA two times a week to three generations of mice. The dyes were mixed with hydrogen peroxide before use. A hair dye containing 2.20 percent PPDA 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.

PPDA and PPDA and hydrogen peroxide mixtures were negative in the Ames *Salmonella*/mammalian-microsome mutagenicity test without metabolic activation. Both positive and negative results with metabolic activation have been reported. Different reseachers have used different solvents for the PPDA, different chemicals for induction, different S-9s, and slight modifications to the Ames test procedure. Any or all of these may explain the observed differences in results. Several oxidation products of PPDA were positive in the Ames test.

The urine of rats that received PPDA intraperitoneally three times a week for 8 weeks was not mutagenic in the Ames test. The urine of rats that received PPDA/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 to 2.55 percent PPDA; in the Ames test with metabolic activation their urine was not more mutagenic after hair dye application.

The mutagenic potential of PPDA has been investigated in a variety of other short-term tests. Purified PPDA was not mutagenic to *D. melanogaster*, although an impure sample was mutagenic. PPDA was not mutagenic in the rat micronucleus test after oral administration of two 500 mg/kg doses. PPDA was inactive in the mouse micronucleus test. Oral administration of 200 mg/kg PPDA to male mice depressed testicular DNA synthesis. PPDA was not active at intraperitoneal doses of 5 to 20 mg/kg per day for 5 days in the mouse sperm-head abnormality test. PPDA was negative in a rat hepatocyte primary culture/DNA repair test. Positive results were obtained for PPDA in the mouse lymphoma forward mutation assay. PPDA, in doses of 1.85 to 3.2 μg/5.2 $\times$ 10^4 cells, was positive in a test measuring the survival of rat embryo cells.

PPDA in the feed of rats and mice at concentrations of 625 and 1250 ppm for 103 weeks was not carcinogenic. There was no evidence of a carcinogenic effect after the oral administration of 0.06 to 30 mg/kg per day PPDA for 8 months to small numbers of rats. PPDA was not carcinogenic in assays in which 5 and 10 percent solutions were applied topically twice a week in doses of 0.02 ml to mice for their lifetime and to female rabbits for 85 weeks.

Three hair dyes containing 1.5 percent PPDA were mixed with hydrogen peroxide before use, and 0.05 ml was applied topically to mice weekly or every 2 weeks for 18 months; carcinogenic activity was not observed. No evidence of a carcinogenic effect was found after the topical administration of 0.5 ml weekly

for 2 years to mice of two hair dyes containing 1.5 percent PPDA and mixed with hydrogen peroxide immediately before use. No carcinogenic effects were observed when four hair dye composite formulations containing 1 to 4 percent PPDA were mixed with hydrogen peroxide and 0.025 ml of the dyes were applied topically weekly for 21 to 23 months to mice. Three hair dye formulations containing 2 to 4 percent PPDA 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 years. An increase in pituitary adenomas was observed in the rats receiving the 4 percent formulation; these adenomas have a high background incidence in rats.

The primary irritation index for 50 percent PPDA applied to the skin of 6 human volunteers for 24 hours under occlusive conditions was 0.8 of a maximum possible total of 8. All of 24 subjects were sensitized after five 48-hour induction patches of 10 percent PPDA. Ten 48- or 72-hour occluded patches with 0.01 percent PPDA resulted in sensitization in 7 of 97 (7.2 percent) human volunteers, with 0.10 percent PPDA resulted in sensitization in 11 of 98 (11.2 percent), and with 1 percent PPDA resulted in sensitization in 47 of 88 (53.4 percent). A maximization test using 2 percent PPDA for induction sensitized 15 of 34 (44 percent) volunteers. A 10 percent aqueous solution of a dye composition containing 2 percent PPDA was used for nine 24-hour induction patches; at challenge, significant dermatitis was observed in 7 of 22 (31.8 percent) of the volunteers. Repeated insult patch tests were conducted on 206 subjects with four hair dyes containing up to 2.144 percent PPDA and 0.049 percent 4NOPD; the hair dyes did not cause irritation or sensitization.

A variety of patch tests with PPDA has been performed on subjects from a variety of populations. Many of these reports are of diagnostic patch tests performed on clinical subjects suffering from skin disease; positive reactions varied from 1.1 to 84.6 percent. A PPDA photopatch test was conducted on 1 subject; PPDA was not phototoxic.

Positive reactions to patches with two hair dyes after mixing them with hydrogen peroxide varied from 15.4 to 100 percent. A beautician developed generalized urticaria when working with hair dyes.

Dermatitis was observed in a man who had dyed his own hair; patch tests were positive for PPDA, the hair dye, and his own hair. Two men had positive patch tests to PPDA and their wives' dyed hair. A third man's eczema flared up when his wife had just dyed her hair; patch tests to PPDA and the hair dye were positive and to hair dyed a week previously was negative. Twenty subjects with strongly positive reactions to PPDA were patch tested with hair dyed with a PPDA-containing formulation; there were no positives.

Edema of the face, neck, ears, and scalp has occurred after hair dye use. Edema of the eyelids and conjunctiva and tearing have been observed and more severe reactions have occurred after the application of PPDA-containing hair dyes to the eyebrows and eyelashes. Vision can be lost or permanently damaged.

A variety of epidemiological studies assess whether occupational exposure to and use of hair dyes increases the risk of cancer. These studies have not produced evidence of any carcinogenic effect from hair dyes on the organs investigated among the occupations and users examined.

DISCUSSION

PPDA may or may not cause mutations depending on the test system and test conditions. In the Ames test, different researchers used different solvents for the PPDA, different chemicals for induction, different metabolic activation systems, and slight modifications of the test procedure; any or all of these may explain the observed differences in results. Most researchers reported that PPDA was not teratogenic or carcinogenic.

Application of hair dyes containing PPDA to the eyebrows and eyelashes can result in lost or permanently damaged vision. PPDA is a sensitizer for guinea pigs and for human beings. Phototoxicity and photosensitization data are not available. Hair dyes containing PPDA are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal Food, Drug and Cosmetic Act of 1938 when cautionary statements and patch test instructions are conspicuously displayed on the labels. Prophetic patch testing of hair dye formulations with open patches is less predictive of skin reactions than patch testing with closed patches; false negative reactions may occur. Some persons may be sensitized even under the proper conditions of use.

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.

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p-PHENYLENEDIAMINE

A safety assessment on *p*-Phenylenediamine was published in 1985 in which the CIR Expert Panel acknowledged that *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 concluded that *p*-Phenylenediamine is safe as a hair dye ingredient at the current concentrations of use (Elder 1985). Studies available since that safety assessment was completed, along with updated information regarding uses and use concentrations, were considered by the CIR Expert Panel. The Panel determined to not reopen the safety assessment.

Although the safety of *p*-Phenylenediamine as a hair dye ingredient was reaffirmed, the Panel did agree with FDA that other uses of this dye are unapproved. The Panel expressed particular concern over the practice of combining *p*-Phenylenediamine with henna (so-called dark henna) for use in temporary tattoos—*p*-Phenylenediamine is a known sensitizer, highly inappropriate for such use 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 (for more information, see the FDA website at http://www.cfsan.fda.gov/~dms/costatt.html). The Panel also will work with the Consumer Federation of America to help the public understand the need to avoid using such unapproved and potentially dangerous products.

The CIR Expert Panel also reviewed hair dye epidemiology data. In 1993, an International Agency for Research on Cancer (IARC) working group evaluated 78 epidemiology literature citations and concluded that "personal use of hair colourants cannot be evaluated as to its carcinogenicity" and that occupation as a hairdresser or barber entails exposures that are probably carcinogenic" (IARC 1993). The IARC report did not distinguish between personal use of oxidative/permanent versus direct hair dyes, or distinguish among the multiple chemical exposures in addition to hair dyes to which a hairdresser or barber might be exposed.

In 2003, an updated review of the available epidemiology literature was prepared (Helzlsouer et al. 2003). This review considered 83 literature citations available since the IARC review. The authors found insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers.

In considering this information, the CIR Expert Panel agreed that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other end points described in the Helzlsouer et al. (2003) review.

The Panel also stated that use of direct hair dyes, although not the focus in all investigations, appear to have little evidence of an association with adverse events as reported in epidemiology studies. However, direct hair dyes are a diverse group of chemicals and the determination of safety may hinge on other safety test data.

p-Phenylenediamine was used in 500 hair-coloring products in 1981, at concentrations of ≤0.1% to 5%. In 2002, *p*-Phenylenediamine was used in 1178 hair-coloring products and in 2 nail care products. Use concentration data provided in 2004 indicated use at concentrations of ≤0014% to ≤4% in hair coloring products. The 2004 use concentration data were provided by CTFA (CTFA 2004).

Available use and concentration information is shown in Table 16. The most recent information now constitutes the present practices of use.

TABLE 16
Historical and current cosmetic product uses and concentrations for *p*-Phenylenediamine

Product category	1981 uses (Elder 1985)	2002 uses (FDA 2002)	1981 concentrations (Elder 1985) %	2004 concentrations (CTFA 2005) %
Hair coloring				
Dyes and colors	493	1167	≤0.1–5	≤4
Tints	7	9	≤0.1	—
Rinses	—	—	—	≤0.0014
Color sprays	—	1	—	—
Lighteners with color	—	1	—	—
Nail care				
Basecoats and undercoats	—	2	—	—
Total uses/ranges for *p*-Phenylenediamine	**500**	**1180**	**≤0.1–5**	**≤0.0014–≤4**

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PHENYL TRIMETHICONE

In 1986, the CIR Expert Panel found that Phenyl Trimethicone is safe as a cosmetic ingredient in the present practices of use and concentration (Elder 1986). A review of the recent literature uncovered no new studies regarding Phenyl Trimethicone,

Amended Final Report of the Cosmetic Ingredient Review Expert Panel

Safety Assessment of p-Phenylenediamine, p-Phenylenediamine HCl, and p-Phenylenediamine Sulfate

December 11, 2007

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Amended Final Report of the Safety Assessment of p-Phenylenediamine, p-Phenylenediamine HCl, and p-Phenylenediamine Sulfate

Abstract: p-Phenylenediamine is an aromatic amine that has been used in oxidatve/permanent hair dyes for over 100 years. It is mixed with hydrogen peroxide immediately before use. Almost 1500 hair-coloring products contain p-Phenylenediamine at concentrations from 2 - 4%. The extent of use of p-Phenylenediamine HCl and p-Phenylenedianmine Sulfate is not known, but use concentrations are around 6%. p-Phenylenediamine for use in hair dyes is manufactured using direct nitration of benzene without chlorinating. This method does not yield chlorinated compounds such as chloro- and dichloroanilines or aminobiphenyls. Following dermal administration of radiolabeled p-Phenylenediamine to rats, the metabolite N,N'-diacetyl-p-Phenylenediamine was detected in plasma, suggesting that topically applied p-Phenylenediamine is metabolized in the skin. That metabolite plus monoacetyl-p-Phenylenediamine were reported as urinary metabolites in human studies. p-Phenylenediamine administered orally, intraperitoneally, or subcutaneously to mice, rats, and rabbits was widely distributed, metabolized to several derivatives, and rapidly excreted in the urine and feces. The acute oral LD_{50} of p-Phenylenediamine for rats ranged from 80 to 98 mg/kg. The acute intraperitoneal LD_{50} of an aqueous p-Phenylenediamine solution for rats was 37 mg/kg. The percutaneous LD_{50} for rabbits of a hair dye composite containing 1.2% p-Phenylenediamine was greater than 10 g/kg. Chronic topical administration of 10% p-Phenylenediamine solutions was not toxic to mice and rabbits. Subchronic and chronic dermal administration of hair dye products containing up to 4% p-Phenylenediamine was not toxic to mice, rabbits, and rats. At single oral doses up to 80 mg/kg, p-Phenylenediamine did not exert a primary effect on the nervous system of rats. Rhabdomyolysis was observed in mice dosed orally with 35 mg/kg and 70 mg/kg p-Phenylenediamine and in dogs at doses up to 100 mg/kg. 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. 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. In one study, p-Phenylenediamine was a guinea pig sensitizer at induction concentrations as low as 0.001%, but was not a sensitizer in another study of a haircoloring formulation containing 2% p-Phenylenediamine. Cross-reactivities to p-Phenylenediamine were confirmed in guinea pigs challenged with p-toluenediamine ·HCl, p-aminophenol, p-aminoazobenzene, and Sudan III in the maximization test, but not with 4-N,N-diethyl-2-methyl-1,4-phenylenediamine · HCl or 4-(N-ethyl-N-2-methan-sulphonamidoethyl)-2-methyl-1,4-phenylenediamine · 1.5 H_2SO_4 · H_2O. In animal tests, p-Phenylenediamine was, at most, moderately irritating when instilled into the eyes. p-Phenylenediamine was not a reproductive or developmental toxicant in several animal tests. p-Phenylenediamine, with or without hydrogen peroxide, was negative in the Ames Salmonella/mammalian-microsome mutagenicity test without metabolic activation; with metabolic activation, both positive and negative results have been reported. Several oxidation products of p-Phenylenediamine were positive in the Ames test. Purified p-Phenylenediamine was not mutagenic to *D. melanogaster*. p-Phenylenediamine was not mutagenic in the rat or mouse micronucleus test. p-Phenylenediamine administered orally to male mice depressed testicular DNA synthesis, but p-Phenylenediamine was not active 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. The urine of rats that received p-Phenylenediamine intraperitoneally was not mutagenic in the Ames test. The urine of rats that received p-Phenylenediamine/resorcinol conjugates topically was mutagenic with metabolic activation and was not mutagenic without metabolic activation. The urine from women who used hair dyes containing p-Phenylenediamine was not mutagenic with metabolic activation in the Ames test. Similar genotoxicity results were reported for p-Phenylenediamine HCl in a wide variety of genotoxicity assays. p-Phenylenediamine in the feed of rats and mice at concentrations of 625 and 1250 ppm for 103 weeks was not carcinogenic. Several other studies in rats and mice produced similar findings. One study in female rats with both topical application and subcutaneous injection of oxidized p-Phenylenediamine HCl for 18 months resulted in a statistically significant increase in the incidence of mammary gland tumors and uterine tumors and soft tissue tumors of both malignant and benign types. In another study using rats, an increase in pituitary adenomas was observed. p-Phenylenediamine is considered to be a human skin sensitizer, with less sensitization at lower concentrations. p-Phenylenediamine was not phototoxic in limited human testing. Numerous case reports of dermatitis following so-called dark (black) henna tattoo application have also been identified; p-Phenylenediamine is one of the additives that is used to accelerate drying and darken the reddish color of the henna in dark henna products. Edema of the face, neck, ears, and scalp and depigmentation have been reported after hair dye use. Edema of the eyelids and conjunctiva and tearing have been observed and more severe reactions (damage to vision) have occurred after the application of p-Phenylenediamine-containing hair dyes to the eyebrows and eyelashes. A summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml. In considering hair dye epidemiology data, the CIR Expert Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other endpoints, based on lack of strength of the associations

and inconsistency of findings. The CIR Expert Panel noted that 4-Aminobiphenyl and 2-aminobiphenyl have been detected in batches of chemical research grade p-Phenylenediamine (purity of 97%), presumably as a by-product of synthesis via reduction of p-nitroaniline. The major U.S. manufacturer of p-Phenylenediamine produces this p-Phenylenediamine at a purity of > 99% for use in hair dyes via the process of direct nitration of benzene without chlorinating, which does not yield aminobiphenyl compounds. It is the expectation of the CIR Expert Panel that 99% pure p-Phenylenediamine (free of aminobiphenyls) is being and will continue to be used by the cosmetics industry. Because of the dangers involved, hair dyes containing p-Phenylenediamine should not be applied to the eyebrows and eyelashes and consumers should not use dark henna tattoos. By Federal law, coal tar hair dye products, including those containing p-Phenylenediamine, are exempt from the principal adulteration provision and the color additive provisions of the Federal Food, Drug and Cosmetic Act of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. While some persons may be sensitized under proper conditions of hair dye use, the Expert Panel expects that following label instructions will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures. The CIR Expert Panel concluded that p-Phenylenediamine, p-Phenylenediamine HCl, and p-Phenylenediamine Sulfate are safe as hair dyes in the practices of use and concentration as described in this safety assessment.

INTRODUCTION

An earlier safety assessment of p-Phenylenediamine(Elder 1985) acknowledged that p-Phenylenediamine is a sensitizer and that some persons may be sensitized under intended conditions of use, but that for persons not sensitized, concluded that this ingredient is safe as a hair dye ingredient at the current concentrations of use. CIR undertook to amend that safety assessment to include the hydrochloride and sulfate salts of p-Phenylenediamine.

CHEMISTRY

Definition and Structure

p-Phenylenediamine

As given in the *International Cosmetic Ingredient Dictionary and Handbook*, p-Phenylenediamine (CAS No. 106-50-3 is an aromatic amine that conforms to the structure shown in Figure 1 (Gottschalck and McEwen 2006).

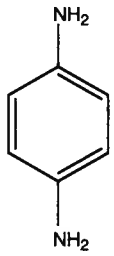

Figure 1. Chemical structure of p-Phenylenediamine.

Synonyms listed by Estrin et al. 1982; Greenberg and Lester 1954; Hawley 1971; International Agency for Research on Cancer [IARC] 1978; Sax 1979; The Society of Dyers and Colourists 1971; Windholz 1976; and Gottschalck and McEwen 2006 include:

- p-Aminoaniline;
- 1,4-Benzenediamine;
- CI 76060;
- p-Diaminobenzene;
- Oxidation Base 10;
- 1,4-Phenylenediamine;
- p-Benzenediamine;
- 4-Aminoaniline;
- 1,4-Diaminobenzene;
- Paradiaminobenzene; and
- PPD.

Trade names listed by Gottschalck and McEwen (2006) include:

- BASF Ursol D;
- Benzofur D;
- Colorex PPD-CG;
- Covastyle PPD;
- Developer PF;
- Durafur Black R;
- Fouramine D;
- Fourrine 1 and Fourrine D;
- Fur Black 41867;
- Fur Brown 41866;
- Furro D;
- Fur Yellow;
- Futramine D;
- Jarocol PPD;
- Nako H;
- OriStar PPD;
- Orsin;
- Pelagol D, Pelagol DR and Pelagol;
- Grey D;
- Peltol D;
- Renal PF;
- Santoflex LC;
- Tertral D;
- Ursol D;
- Zoba Black D;
- Rodol D and Rodol D-99;
- C.I. 76060;

- C.I. Developer 13; and
- C.I. Oxidation Base l0.

p-Phenylenediamine HCl

p-Phenylenediamine HCl (CAS Nos. 624-18-0) is the aromatic amine salt that conforms to the structure shown in Figure 2 (Gottschalck and McEwen 2006).

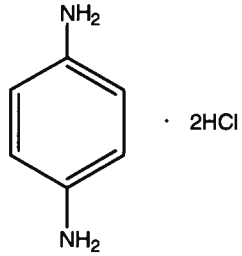

Figure 2. Chemical structure of p –Phenylenediamine HCl

Other names for this chemical include:
- 1,4-Benzenediamine Dihydrochloride;
- CI 76061;
- 1,4-Diaminobenzene Dihydrochloride;
- Oxidation Base 10A;
- p-Phenylenediamine Dihydrochloride; and
- 1,4-Phenylenediamine Hydrochloride.

Rodol DC is a trade name of p-Phenylenediamine HCl (Gottschalck and McEwen 2006).

p-Phenylenediamine Sulfate

p-Phenylenediamine Sulfate (CAS No. 16245-77-5) is the aromatic amine salt that conforms to the structure shown in Figure 3 (Gottschalck and McEwen 2006).

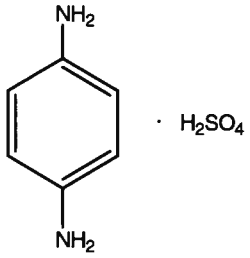

Figure 3. Chemical structure of p-Phenylenediamine Sulfate

Other names include 1,4-Benzenediamine Sulfate and 1,4-Benzenediamine Sulfate (1:1); trade names include Colorex PPDS; Covastyle PPDS; Jarocol PPDS; and Rodol DS (Gottschalck and McEwen 2006).

Chemical and Physical Properties

Aromatic amines, such as p-Phenylenediamine, are nonpolar bases that are readily converted to highly water-soluble hydrochloride salts. Whereas the salts of aromatic amines are relatively stable (IARC 1978), free aromatic amines are usually quite unstable to light, heat, and oxygen and oxidize to colored quinoneimines, quinones, and various

polymerized products (Radomski 1979). 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 (Radomski 1979). According to the Personal Care Products Council (Council), current thinking is that the oxidation products are trapped rather than covalently bonded because there is no further color change that would be expected by reaction with a sulfhydryl group (Council 2008).

Data on the chemical and physical properties of p-Phenylenediamine are presented in Table 1 based on information from Greenberg and Lester 1954; Hawley 1971; IARC 1978; Sax 1979; Windholz 1976;; Beard and Noe 1981; Glabisz and Tomaszewska 1977; Mackison et al. 1978; Pitter and Radkova 1974; The Society of Dyers and Colourists 1971; Weast 1978; and the American Conference of Governmental Industrial Hygiensists [ACGIH] 2000.

According to Elder (1985), 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.

Chemical and physical properties of p-Phenylenediamine HCl and p-Phenylenediamine Sulfate also are included in Table 1.

Reactivity

Furia (1972) stated that phenylenediamine compounds are potent antioxidants. The International Agency for Research on Cancer (IARC) suggested that phenylenediamines and their oxidation products are highly reactive substances that would be expected to react with tissue nucleophiles, causing various biological effects (IARC 1978).

Elder (1985) noted that p-Phenylenediamine 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 trichloride ($FeCl_3$) and 3% hydrogen peroxide (H_20_2) 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.

p-Phenylenediamine Oxidation Products

According to Corbett (1972) and Corbett and Menkart (1973), the oxidation of p-Phenylenediamine (A in Figure 4) by molecular oxygen results initially in the formation of p-benzoquinone diimine (B), which. may react to give either a polymer of the diimine (C) or Bandrowski's base (D).

p-Benzoquinone diimine may also undergo hydrolysis to form monoimine (E), and then undergo further hydrolysis to

Table 1. Chemical and physical properties of p-Phenylenediamine and its hydrochloride and sulfate salts.

Property	Value	Reference
	p-Phenylenediamine HCl	
Appearance	White to gray or pink-beige powder	COLIPA 2006
Molecular weight	181.07	COLIPA 2006
Octanol/water partition coefficient (Log P_{ow})	Calculated: -0.3 ; Experimental: -0.84	COLIPA 2006
Melting point	140.7°C	COLIPA 2006
Solubility (g/100 ml - 22°C for 24 h)	Water ($10 \leq S \leq 20$; ethanol ($S < 10$); DMSO ($S < 1$)	COLIPA 2006
	p-Phenylenediamine Sulfate	
Form	off-white to gray powder	Keystone Aniline Corporation 1999
Molecular weight	206.22	Keystone Aniline Corporation 1999
Solubility	Slightly soluble in water (at 25°C and 60°C); insoluble in isopropyl alcohol (at 25°C and 60°C)	Keystone Aniline Corporation 1999
	p-Phenylenediamine	
Formula	$C_6H_8N_2$	Weast 1978
Molecular weight	108.15	Weast 1978
Boiling point	267°C	Greenberg and Lester 1954; Hawley 1971; IARC 1978; Sax 1979; Windholz 1976; Environmental Protection Agency (EPA) 1982; ACGIH 2000; Beard and Noe 1981; Mackison et al. 1978; Weast 1978; Grasselli 1973
Melting point	139°C	Greenberg and Lester 1954; Beard and Noe 1981
	140°C	IARC 1978; Weast 1978; Grasselli 1973; Grant 1974
	141°C	Mackison et al. 1978
	145 -147°C	Hawley 1971; Sax 1979; Windholz 1976; EPA 1982; ACGIH 2000
Solubility	Water: 3.8% at 24°C	Beard and Noe 1981
	Slightly soluble in water (at 25°C); soluble in water (at 60°C); slightly soluble in isopropyl alcohol (at 25°C and 60°C)	Keystone Aniline Corporation 1999
Volatility (technical product)	< 1 mm at 21°C	IARC 1978; E.I. Du Pont De Nemours and Company 1977
Vapor density	3.72	Sax 1979
Flash point (closed cup)	155.5°C	Hawley 1971; Sax 1979; ACGIH 2000; Mackison et al. 1978
Octanol/water partition coefficient (Log P_{ow})	-0.25	EPA 1982; EPA 1980
	0.2	Wolfram and Maibach 1985
Other partition coefficients	4.0 (intact guinea pig stratum corneum/water); 7.3 (delipidized guinea pig stratum corneum/water).	Wolfram and Maibach 1985
UV light absorption	λmax: 246 nm ($E_1^1 = 788$);315 nm ($E_1^1 = 184$); 310 nm	IARC 1978; Weast 1978; Grasselli 1973; Baranowska et al. 2002
Molecular weight	108.14	Keystone Aniline Corporation 1999

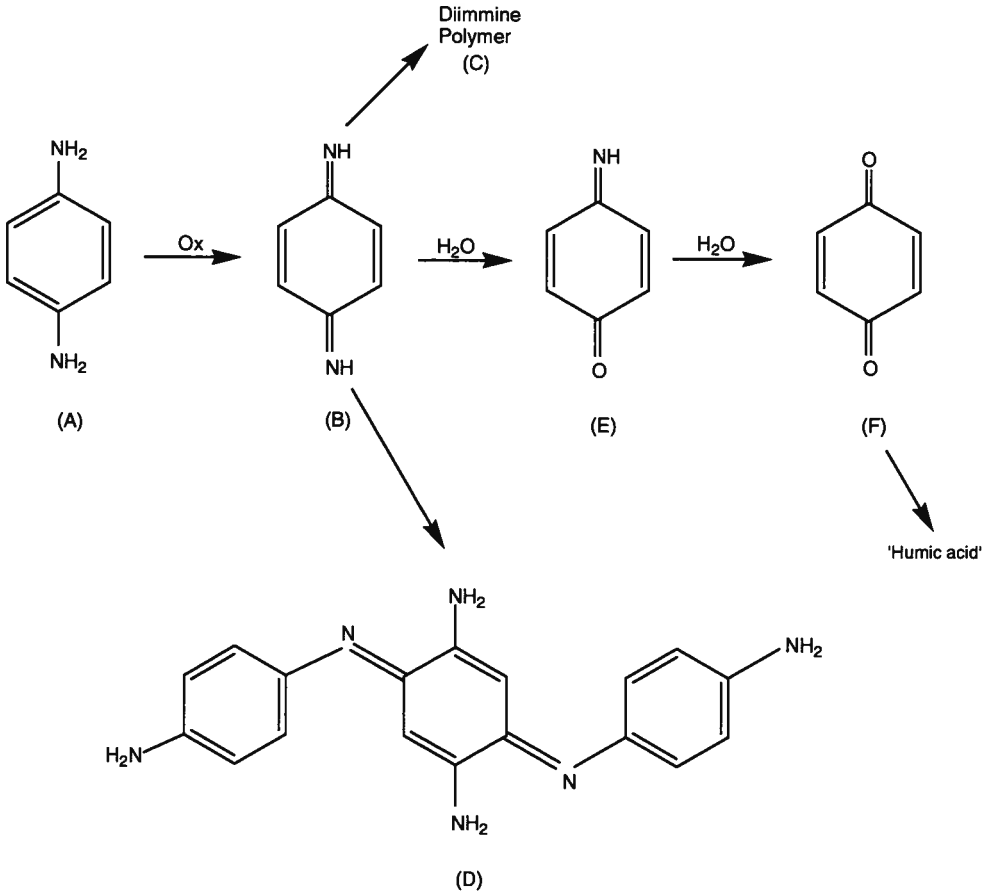

Figure 4. Major pathways for p-Phenylenediamine (A) oxidation where the chemical structures are: B, p-benzoquinone diimine; E, p-benzoquinone imine; F, p-benzoquinone; and D, a specific diimine polymer known as Bandrowski's base.

p-benzoquinone (F) and its decomposition product, humic acid. Nitroaniline and 4,4'-diaminoazobenzene (DAAB) have also been identified as minor oxidation products.

The hydrolysis of p-benzoquinone diimine to p-benzoquinone is only significant at a pH of less than 3 in the presence of a strong oxidizing agent, such as potassium ferricyanide, potassium dichromate, or ferric chloride. At a pH of greater than 9, the formation of p-benzoquinone again is significant, but only if the p-Phenylenediamine solution is at a concentration of less than 0.001%. Polymerization of p-benzoquinone diimine occurs at a pH greater than 9 when a p-Phenylenediamine solution (> 10^{-3} %) is added to a solution of a strong chemical oxidant. The major reaction product under "most relevant conditions" (pH of 3 to 10 and p-Phenylenediamine concentrations of > 10^{-3} %) is the specific diimine polymer Bandrowski's base (Fig. 4 D) (Corbett 1972; Corbett and Menkart 1973).

According to the Scientific Committee on Consumer Products (SCCP), the reactions shown in Figure 4 are examples of self-coupling. As the rate of self-coupling is a very slow reaction compared to the reaction with a coupler in the reaction mixture, Bandrowski's Base may not be formed in an oxidative hair dye mixture containing both p-Phenylenediamine and a coupler (SCCP 2005).

Oxidative Hair Coloring Process

In oxidative (permanent) hair coloring systems, the colored material is produced inside the hair fiber by oxidation of colorless intermediates (Corbett and Menkart 1973). To accomplish the color-forming reaction, 3 classes of chemical reactants are required: a primary intermediate, an oxidant, and a coupler.

Frequently employed intermediates are aromatic o- or p-diamines or aminophenols to produce colors as listed in Table 2 (Corbett 1973). According to Reiss and Fisher (1974), the major primary intermediate used in the US for

permanent hair dyes is p-Phenylenediamine.

Corbett (1973) noted that primary intermediates are capable of undergoing oxidation to form color benzoquinone imines, the "essential reactive species" in the color-forming reaction as shown in Figure 4.

The second necessary component is the oxidant. Hydrogen peroxide is the most frequently used oxidant, although various acids of solid organic hydrogen peroxide adducts are used depending on the hair dye product. Hydrogen peroxide is widely employed because it is a relatively unreactive oxidant and causes a slow oxidation of the primary intermediate in the dye bath.

Table 2. Colors Produced by Primary Intermediates (Burnett and Corbett 1977; Corbett 1976).

Compound	Color on Hair
p-Phenylenediamine	Dark brown
p-Toluylenediamine	Light reddish brown
p-Aminodiphenylamine	Dark gray-black
p-Aminophenol	Light auburn
2-Amino-5-Hydroxytoluene	Golden blond
5-Amino-2-Hydroxytoluene	Reddish blond
o-Aminophenol	Deep gold

The third component necessary for color development is the coupler. Examples of colors as a function of coupler are given in Table 3. By virtue of their strong electron-donating groups, couplers react with the electrophilic quinoneimines to produce leuko-indo dyes. When mixtures of couplers are used, the amount of each dye formed depends on the relative concentration of the couplers in the dye bath, the rate of coupler diffusion into the hair fiber, and the relative reactivities of the couplers at the prevailing pH,.as shown in Table 4.

This author suggested that fading of highly colored indo dyes to light brown shades involves addition of aromatic moieties to the dinuclear indo dye. High humidity has an accelerating effect on both this type of fading and on the intramolecular cyclization of aminoindamines, which results in fading to red shades (Corbett 1973).

Some of the important color-forming reactions of p-Phenylenediamine are presented in Figure 5 (Burnett and Corbett 1977; Corbett 1976), and the chemistry of these reactions is summarized in Figure 6 (Burnett and Corbett 1977; Corbett 1976).

The initial reaction involves oxidation of p-Phenylenediamine by the oxidant, or by oxygen formed by decomposition of the oxidant inside the hair fiber, to give p-benzoquinone diimine. p- Benzoquinone diimine in the form of its conjugate acid then reacts with the coupler and/or unoxidized p-Phenylenediamine to yield a leuko-indo dye. Reaction occurs by electrophilic attack on the most nucleophilic site of the benzene ring of the coupler.

Table 3. Colors Produced by p-Phenylenediamine in the Presence of Various Couplers (Burnett and Corbett 1977; Corbett 1976).

Coupler	Color in Hair
None	Dark Brown
m-Phenylenediamine	Bluish purple
2,4-Diaminoanisole	Purple-blue
m-Aminophenol	Light brown
4-Methyl-3-Aminophenol	Light brown
m-Methoxyphenol	Magenta
6-Methyl-3-Aminophenol	Magenta
2,5-Xylenol	Bluish purple
Resorcinol	Greenish brown
Hydroquinone	Light gray-brown
Catechol	Gray-brown

Table 4. Reactivity of Commonly Used Couplers Toward p-Benzoquinone Diimine (see Figure 4) (Corbett 1973).

Coupler	Experimental Second Order k at 30°C and pH 9.5[a]
Resorcinol	1.5×10^5
m-Aminophenol	5.5×10^4
2,4-Diaminoanisole	6.0×10^4
1-Naphthol	7.4×10^5
p-Phenylenediamine	34.7

[a] d[dye]/dt = k[diimine] [coupler]

If the reactive site on the coupler bears a methoxy group, the indo dye is formed nonoxidatively by elimination of methyl alcohol from the coupled intermediate. Some of these indo dyes are the final colored product in the hair, whereas others undergo further reaction to form polymeric indo compounds (Corbett and Menkart 1973; Corbett 1973; Reiss and Fisher 1974; Burnett and Corbett 1977; Corbett 1976; Brown and Corbett 1979).

Resorcinols react with p-benzoquinone diimine to give a

green trinuclear dye and/or a brown polymeric indoaniline (Corbett 1973; Shah et al. 1972). m-Diamines couple with

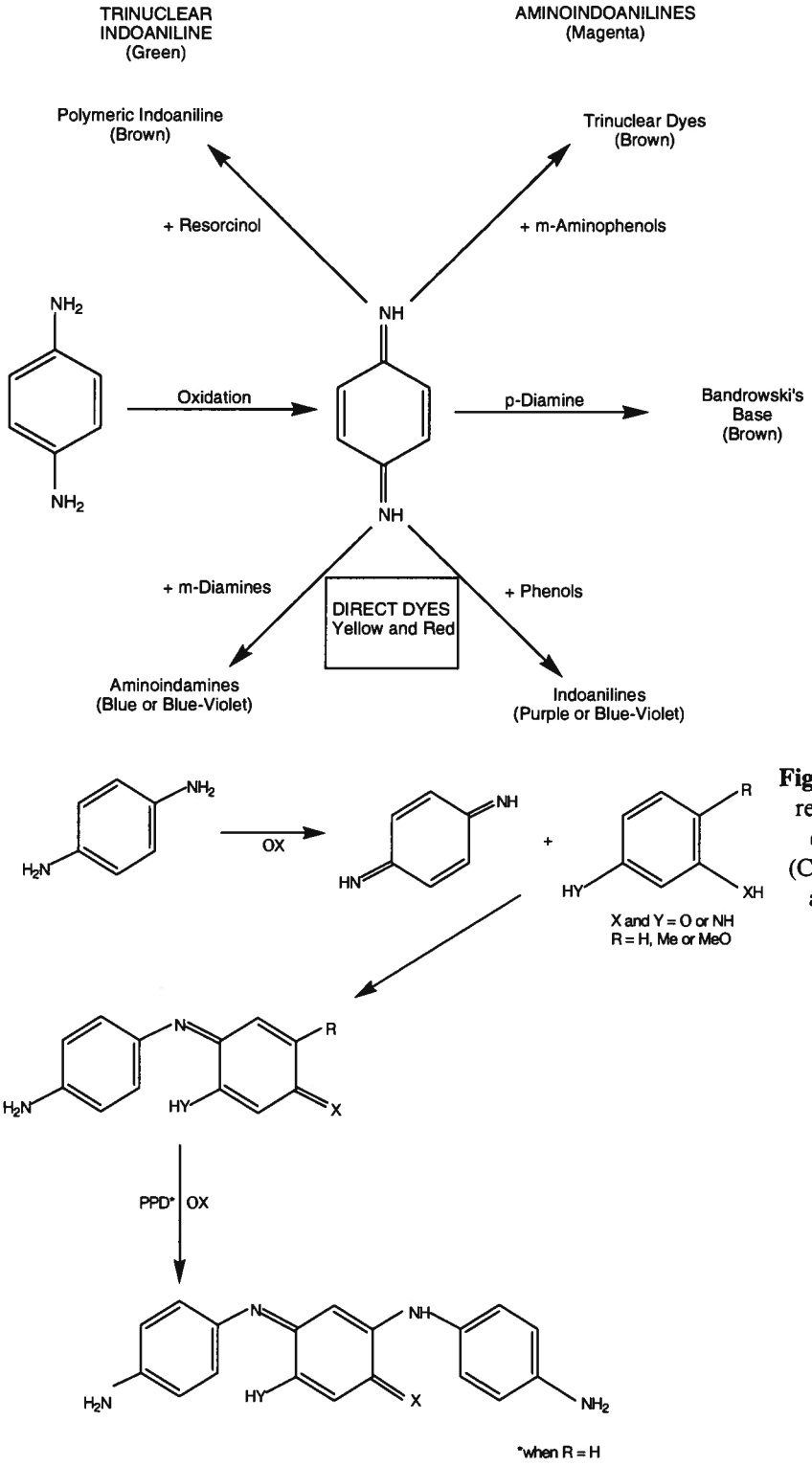

Figure 5. Color-forming reactions in oxidative color development (Corbett 1976; Burnett and Corbett 1977).

Figure 6. The chemistry of oxidative coupling reactions (Corbett 1976; Burnett and Corbett 1977).

p-benzoquinone to yield blue 2-aminoindamines.

Except for the methoxy derivatives, 2-aminoindamines have poor color stability and undergo intramolecular cyclization to red 2,8-diaminophenazines. p-Benzoquinone diimine couples with m-aminophenols at the position para to the hydroxy group to give magenta 2-aminoindoanilines, or, if this position is blocked, coupling occurs para to the amino group to yield magenta 2-hydroxyindamines. p-Phenylenediamine can also react with 2-aminoindoaniline to give a brown triangular dye. The magenta dyes are relatively unstable and fade to a brown species. Coupling of phenols with p-benzoquinone diimine yields purple indoanilines, whereas reaction of p-benzoquinone with p-diamines gives the brown dye, Bandrowski's base (Corbett 1973).

Although the initial oxidation product of p-Phenylenediamine is usually p-benzoquinone diimine (with possible involvement of a free radical intermediate), the nature of the final products of the reaction is dependent on the concentration of the diimine, the nature of the oxidizing agent, the pH of the reaction environment, the presence of coupling agents, and the presence of catalysts or catalytic surfaces.

The oxidation of p-Phenylenediamine by hydrogen peroxide to form p-benzoquinone diimine is relatively slow and even incomplete after 24 hours, whereas the reaction of the coupler with p- benzoquinone diimine is so rapid as to prevent any appreciable buildup of the quinone-imine intermediate, and to prevent completely the formation of Bandrowski's base in the dye solution (although the base may well form in the hair). The half-life of p-benzoquinone diimine is on the order of a few milliseconds, and its concentration under hair dyeing conditions never reaches a detectable level (Corbett and Menkart 1973; Burnett and Corbett 1977; Corbett 1976).

Rastogi et al. (2006) conducted a study to estimate consumer exposure to precursors and couplers of oxidative hair dyes during and after hair dyeing. The concentrations of unconsumed precursors and couplers in 8 hair dye formulations for non-professional use were studied under conditions that reflected hair dyeing. Six products were used to study oxidative hair dye formation in the absence of hair. Significant amounts of unconsumed precursors and couplers remained in the hair dye formulations after final color development; this was true in both the presence and absence of hair. Up to 1.1% p-Phenylenediamine was found in the hair dye formulation after the required color was developed.

Analytical Methods

p-Phenylenediamine

The Association of Official Analytical Chemists has published both a gravimetric method and an iodometric titration method for the determination of p-Phenylenediamine in hair dyes (Horwitz 1970).

Calorimetric methods have been used to analyze aromatic amines, including p-Phenylenediamine, by their reaction with 2,6-xylenol (Corbett 1975), sodium chlorite (Corbett 1975) ruthenium trichloridetriphenylphosphine (Hashmi et al. 1969), thiotrithiazyl chloride (Levin et al. 1967), or peroxydisulfate (Gupta and Srivastava 1971), or by their coupling with diazotized sulphanilic acid and other compounds (Legradi 1967).

A spot test for the detection of p-Phenylenediamine in hair dyes uses a vanillin-isopropyl alcohol reagent (Fregert 1972; Lange 1966).

An acid-impregnated paper tape technique has also been reported (Pinches and Walker 1980).

Additional methods for the separation and/or determination of p-Phenylenediamine or p-Phenylenediamine derivatives and complexes include:

- high-pressure liquid chromatography (Graffeo and Riggin 1978; Sugden et al. 1978; Turchetto et al. 1980),
- gas and gas-liquid chromatography (Choudhary 1980; Goldstein et al. 1968; Knight 1971; Pinter and Kramer 1967; Walle 1968),
- column chromatography on an anion-exchange resin (W ligand-exchange chromatography) (Funasaka et al. 1969),
- gel-permeation chromatography (Protivova and Pospisil 1974),
- thin-layer chromatography (Goldstein et al. 1968; Kottemann 1966; Legatowa 1973; Lepri et al. 1976; Thielemann 1978; Wisneski 1977; Zelazna and Legatowa 1971)
- thin-layer chromatography and electrophoresis (Bassl et al. 1967; Cozzi et al. 1969; Drost and Reith 1967; Lepri et al. 1974; Srivastava and Dua 1975),
- paper chromatography (Galatik 1972; Matrka and Kroupa 1971; Reio 1970),
- chronopotentiometry (Bamberger and Strohl 1969),
- polarography (Beilis 1965; Usvyatsov et al. 1975),
- titrimetric techniques (Ignaczak and Dziegiec 1975; Ratnikova et al. 1974),
- spectrophotometry (Iordanova 1978; Jenik 1979; Von Mallinckro and Herrmann 1969),
- atomic absorption spectrophotometry (Mitsui and Fugimura 1974),
- nuclear magnetic resonance and mass spectrometry (Hutzinger 1969), and
- thermogravimetric techniques (Lorant 1977).

p-Phenylenediamine Sulfate

p-Phenylenediamine Sulfate has been analyzed by infrared spectroscopy (Keystone Aniline Corporation, 1999).

Method of Manufacture and Impurities

p-Phenylenediamine

According to the Scientific Committee on Consumer Products (SCCP 2006), the purity of both the p-Phenylenediamine free base and its dihydrochloride salt is > 99%. The following impurities of p-Phenylenediamine (reported as specification limits) are also mentioned: o-aminophenol (< 500 ppm), o-Phenylenediamine (< 200 ppm), m-Phenylenediamine (< 200 ppm), and aniline (< 50 ppm).

White et al. (2006) reported that 99.9% pure p-Phenylenediamine has, as its main impurity, Bandrowski's base at 0.1%. Traces of an organic impurity, tentatively identified as 4,4'-azodianiline, were also reported. A 7-month stability analysis indicated an increase in the concentration of Brandrowski's base from 0.1% to 0.5%.

According to CTFA (2007a), p-Phenylenediamine is manufactured using the following three methods: reduction of para-nitroaniline, aniline diazotization, and direct nitration of benzene without chlorinating. The third method does not lend itself to and has not been shown to contain chlorinated compounds such as chloro- and dichloroanilines or aminobiphenyls.

Turesky et al. (2003) reported that some batches of chemical research grade p-Phenylenediamine (purity of 97%) were contaminated with 4-ABP (up to 500 ppb) and 2-ABP (up to 70 ppm) and may be the source of ABP contamination in hair dyes.

CTFA (2007a) noted that the major U.S. manufacturer of p-Phenylenediamine produces this chemical at a purity of > 99% for use in hair dyes via the process of direct nitration of benzene without chlorinating, which does not lend itself to the formation of aminobiphenyl compounds, and not by the aniline diazotization process.

p-Phenylenediamine HCl

A specification for p-Phenylenediamine HCl includes the following: titre (> 98 g/100g, determined by potentiometry), relative purity (> 99%), aniline (< 100 µg/g), o-aminophenol (< 500 µg/g), o-phenylenediamine (< 200 µg/g), and m-phenylenediamine (< 200 µg/g) (COLIPA 2006).

p-Phenylenediamine Sulfate

According to Keystone Aniline Corporation (1999), the product specifications for p-Phenylenediamine Sulfate are as follows: off-white to gray powder, purity (95.0% minimum), ash (0.5% maximum), iron (50 ppm maximum), and conforms to standard IR spectrum.

USE

Purpose in Cosmetics

As given in the *International Cosmetic Ingredient*

Dictionary and Handbook, p-Phenylenediamine HCl, p-Phenylenediamine Sulfate, and p-Phenylenediamine function as hair colorants in cosmetic products (Gottschalck and McEwen 2006). According to Keystone Aniline Corporation (1999), *p*-Phenylenediamine is categorized as a permanent oxidation dye intermediate, and its primary use is that of a primary intermediate.

Scope and Extent of Use in Cosmetics

Data submitted to FDA in 1981 by cosmetic firms participating in the Voluntary Cosmetic Registration Program (VCRP) indicated that p-Phenylenediamine was used in a total of 500 hair coloring formulations (Elder 1985). Because data were only submitted within the framework of preset concentration ranges, the opportunity for overestimation of the actual concentration of an ingredient in a particular product existed. No uses of the hydrochloride or sulfate salt were reported in 1981.These historical data are given in Table 5, with current useage and use concentration information.

The highest level of p-Phenylenediamine in hair color products has been associated with black shades (normally 3.5 to 4%) (CTFA 1982a).

VCRP use data were provided in 2006 (FDA 2006), and use concentration data were available from a cosmetics industry survey (CTFA 2007b). VCRP data indicate that p-Phenylenediamine was being used in a total of 1497 cosmetic products, 1478 of which are hair dyes and colors. There were no uses of the hydrochloride or sulfate salt reported in the VCRP.

Use concentration data from the Cosmetic, Toiletry, and Fragrance Association (CTFA 2007b) indicate that p-Phenylenediamine was being used in cosmetics at concentrations ranging from 2% to 4% and that both p-Phenylenediamine HCl and p-Phenylenediamine Sulfate were being used at a concentration of 6%.

Permanent hair dye preparations have usually been packaged in 2 containers, one holding the intermediate mixture and the other the oxidizing agent (Reiss and Fisher 1974). Upon product use, the intermediate is diluted and oxidized by mixing with equal parts of the oxidant, usually 6% hydrogen peroxide. This process may bleach the natural hair pigment (Marzulli et al. 1978). The hair dye base usually consists of an ammonium oleate soap with small amounts of detergent. Free ammonia is present to promote the oxidative color reaction and to give an "on-head" pH of approximately 9.5 (Corbett and Menkart 1973). Other materials may be present in the dye preparation; these include reducing agents to control the rate of reaction and various ingredients to aid in penetration, sequestering, foaming, and adhesion (Spoor 1977).

In permanent hair dyes containing p-Phenylenediamine, the reactive ingredients of the formulation penetrate the cortex of the hair where the colored compounds are formed. Color development is complete in 15 to 30 minutes. The dyeing is

Table 5. Historical and current cosmetic product uses and concentrations for p-Phenylenediamine and its hydrochloride and sulfate salts.

Product Category	1981 uses (Elder 1985)	2006 uses (FDA, 2006)	Total products in category (FDA 2006)	1981 concentrations (Elder 1985) (%)	2007 concentrations (CTFA 2007b) (%)
			p-Phenylenediamine		
Hair coloring products					
Dyes and colors	493	1478	1600	≤0.1 to 5	2 to 4 (before dilution; 1-2 after dilution)
Tints	7	16	56	≤0.1	-
Lighteners with color	-	3	14	-	-
Total uses/ranges for p-Phenylenediamine	**500**	**1,497**		**≤0.1% to 5**	**≤0.0014% to ≤4**
			p-Phenylenediamine HCl		
Hair coloring products					
Dyes and colors	-	-	1600	-	6 (before dilution; 3 after dilution)
Total uses/ranges for p-Phenylenediamine HCl	**-**	**-**		**-**	**6**
			p-Phenylenediamine Sulfate		
Hair coloring products					
Dyes and colors	-	-	1600	-	6 (before dilution; 3 after dilution)
Total uses/ranges for p-Phenylenediamine Sulfate	**-**	**-**		**-**	**6**

permanent; the oxidative dye formed with p-Phenylenediamine is fixed in the hair cortex and is not removed by shampooing (Reiss and Fisher 1974). Subsequent dyeing is necessitated primarily by the need to color new hair growth rather than by the fading of the previously colored hair. However, some off-shade fading eventually does occur, as evidenced by the development of a red tinge. This fading is attributed to slow chemical changes in the indo dyes (Corbett and Menkart 1973; Schwartz et al. 1979).

Permanent hair coloring formulations are applied to or may come in contact with hair, skin, eyes, and nails. While hairdressers may come in contact with products containing p-Phenylenediamine several times a day, consumers use hair dyes once every 6 weeks, and maximum exposure to p-Phenylenediamine would occur in users of black oxidation hair dyes. These users would apply approximately 100 g of a dye composition containing up to 2 g of p-Phenylenediamine on each occasion.(CTFA 1982a).

Forty percent of women in the US are estimated to be regular users of hair dyes (Corbett and Menkart 1973). Under normal use conditions, skin contact with the hair dye is restricted to 30 minutes with a solution containing less than 3% p-Phenylenediamine (Corbett and Menkart 1973; Burnett and Corbett 1977; Corbett 1976). Users are exposed to unreacted p-Phenylenediamine and couplers, as well as to reactive intermediates, particularly quinone-imine and the various indo dyes (Burnett and Corbett 1977; Corbett 1976). However, exposure to quinone-imine and the brown dye, Bandrowski's base, may be limited.

Whereas the oxidation of p-Phenylenediamine by hydrogen peroxide to form p-benzoquinone diimine is relatively slow and even incomplete after 24 hours, the reaction of the various couplers with the diimine is so rapid as to prevent any appreciable buildup of the quinone-imine intermediate and to prevent completely the formation of Bandrowski's base in the dye solution (although it may form in the hair). The half-life of p-benzoquinone diimine is on the order of a few milliseconds, and its concentration under use conditions never reaches a detectable level (Corbett and Menkart 1973; Burnett and Corbett 1977; Corbett 1976).

Gagliardi et al. (1992) assessed the rate of exposure to p-Phenylenediamine vapors by hairdressing employees during a work day, considering the type of salon and its characteristics (small, medium, and large), the number of dye applications per day, and the chemical-physical characteristics of the oxidation dyes. The authors concluded that, even under extreme conditions, lung exposure of hairdressing employees to p-Phenylenediamine is not possible.

In the European Union, m- and p-Phenylenediamines, their N-substituted derivatives, and their salts, and, also, N-substituted derivatives of o-Phenylenediamines are approved (at use level/restriction of 6%, calculated as free base) for both general and professional use as oxidizing coloring agents for hair dyeing. The required product label warnings are as follows: Products for professional use - For professional use only. Contains phenylenediamines. Can

cause an allergic reaction. Wear suitable gloves; Products for general use - Can cause an allergic reaction. Contains phenylenediamines. Do not use to dye eyelashes or eyebrows (European Commission 2007).

p-Phenylenediamine and its salt are included on the list of ingredients of quasi drugs that are marketed in Japan (Ministry of Health, Labor and Welfare (MHLW) 2000). By definition, quasi-drugs must have a mild effect on the body, but are neither intended for the diagnosis, prevention, or treatment of disease, nor to affect the structure or function of the body. Hair dyes are among the products that are designated as quasi-drugs by the MHLW (MHLW 2002).

In 2006, the SCCP published a document (references included) containing its opinion on the safety of p-Phenylenediamine as an oxidative hair dye (SCCP 2006). The opinion included:

- p-Phenylenediamine alone is being not genotoxic, but, positive findings from genotoxicity studies *in vivo/in vitro* of p-Phenylenediamine in combination with couplers and/or hydrogen peroxide as well in a carcinogenicity study were reported.
- using accepted approaches, a margin of safety (MOS) of 77 was calculated. In another approach, the AUC in rats following a peroral dosage of 4 mg/kg (corresponding to the NOAEL) was compared to the AUC in humans following application of a hair dye containing ^{14}C-labeled PPD. In this case a safety margin of 16.3 was obtained which was not considered sufficient by the SCCP.
- experimental evidence was provided that PPD is metabolised in the skin to acetylated (i.e. detoxified) derivatives and, furthermore, that presumably activation of p-Phenylenediamine (formation of monoxygenated derivatives) does not occur.

Overall, the SCCP was of the opinion that the information submitted is insufficient to allow a final risk assessment to be carried out. Additional data are required on *in vivo* genotoxicity and/or carcinogenicity of PPD in combination with hydrogen peroxide and couplers (to simulate consumer exposure). Further information also is needed to support the view that the MOS is sufficiently high.

The SCCP also noted that there is an increasing use of hair dyes by young people and additional exposure to PPD-related substances from temporary tattoos and clothing textiles. PPD is an extreme sensitizer and the risk of allergy occurring in the consumer should be realized.

In the United States, p-Phenylenediamine, p-Phenylenedianmine HCl, and p-Phenylenediamine Sulfate are considered coal tar hair dyes for which regulations require caution statements and instructions regarding patch tests in order to be exempt from the principal adulteration provision and from the color additive provision in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 (FDA, 1979).

Product labels shall bear a caution statement and patch test instructions for determining whether the product causes skin irritation. 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.

The FDA has determined that uses of p-Phenylenediamine other than as a hair dye are unapproved, including as one of the additives that is used to accelerate drying and darken the reddish color of the henna in dark (black) henna products.

At its February 11, 1992 meeting, the Cosmetic Ingredient Review (CIR) Expert Panel issued the following policy statement on coal tar hair dye product labeling:

> The Cosmetic Ingredient Review (CIR) Expert Panel has reviewed the cosmetic industry's current coal tar hair dye product labeling, which recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 24 hours after application of the test material and prior to the use of a hair dye formulation.
>
> Since the recommendation on the industry's adopted labeling establishes a procedure for individual user safety testing, it is most important that the recommended procedure be consistent with current medical practice.
>
> There is a general consensus among dermatologists that screening of patients for sensitization (allergic contact dermatitis) should be conducted by the procedures used by the North American Contact Dermatitis Group (NACDG) and the International Contact Dermatitis Group (North American Contact Dermatitis Group, 1980; Eiermann et al., 1982; Adams et al., 1985).
>
> Basically, these procedures state that test material should be applied at an acceptable concentration to the patient, covered with an appropriate occlusive patch, and evaluated for sensitization at 48 and 72 hours after application. The CIR Expert Panel has cited the results of studies conducted by both the North American Contact Dermatitis Group and the International Contact Dermatitis Group in its safety evaluation reports on cosmetic ingredients (Elder, 1985a).

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

No opposition to this recommendation was received. At the February 11, 1992 public meeting of the CIR Expert Panel, this policy statement was adopted.

Noncosmetic Use

p-Phenylenediamine

In addition to its cosmetic use as a dye intermediate in permanent hair coloring formulations, p-Phenylenediamine is used as a photographic developing agent, a laboratory reagent, 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. The compound 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 polyparaphenylene terephthalamide, a fiber used in tire cords (Hawley 1971; IARC 1978; The Society of Dyers and Colourists 1971; Windholz 1976; EPA 1982; Beard and Noe 1981; EPA 1980; Baer et al. 1973; Cundell and Mulcock 1976; Grant 1969; Kersey and Stevenson 1980; Schorr 1974).

In dye manufacturing, p-Phenylenediamine is used as an intermediate in the production of a number of colors having commercial significance (IARC 1978; The Society of Dyers and Colourists 1971; National Cancer Institute [NCI] 1978; The Society of Dyers and Colourists 1956). These include C.I. Direct Orange 27, Disperse Yellow 9, Solvent Orange 53, Sulphur Brown 23, Leuco Sulphur Brown 23, C.I. Disperse Black 2, C.I. Direct Green 28, C.I. Acid Violet 3, C.I. Direct Black 9, C.I. Direct Black 2, C.I. Direct Black 80, and C.I. Direct Black 19. The compound is also an intermediate in the production of dyes derived from 4-aminoacetanilide, 4-aminoformanilide, 4-nitro-aniline, and 4aminooxanilic acid (IARC 1978; The Society of Dyers and Colourists 1971).

Chemical and biochemical applications of p-Phenylenediamine include use as an indicator and reagent for nitrogen (Grant 1969), as a chromogenic spray reagent for thin-layer chromatography (Adamovic 1966), and as a hydrogen donor for peroxidase assay systems (Awasthi et al. 1977; Pilz et al. 1976a,b,c; Pilz et al. 1978).

p-Phenylenediamine is also used for removing nitrogen and sulfur oxides from waste gases (Ito and Tatsumi 1976; Ito et al. 1978; Kohler et al. 1978; Matsumoto and Ukawa 1979; Ukawa and Okino 1979) and for the calorimetric determination of hydrogen sulfide in air (Fedorova 1971; Stan et al. 1979), thiocyanate in biological fluids (Pettigrew and Fell 1972), and inorganic phosphorus in serum (Parekh and Jung 1970). Other applications include use as a substrate to measure the activity of oxidative enzymes (Anderson et al. 1968; Cabrillat and Fontainiere 1980; Dimmit 1975; Hohbadel et al. 1975; Jensen et al. 1977; Kelleher and Mason 1979; Lamand et al. 1980; Linder and Moor 1977; MacDonald et al. 1980; Marcollett et al. 1980; Peisach and Levine 1965; Pettersson 1970), and as a staining agent for biological materials (Colman and Stockert 1979; Esponda and Stockert 1978; Huff et al. 1982; Ingjer 1979; Juhlin and Shelley 1977; Korneliussen et al. 1978; Krauhs and Salinas 1980; Ledingham and Simpson 1970; Ledingham and Simpson 1972; Macbeth et al. 1975; Sheibani et al. 1981; Shepard and Mitchell 1977; Snipes 1977; Stockert 1977; Szent-Gyorgyi 1980; Vaganova and Sekamova 1980).

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. It is also used in the manufacture of rubber and plastics (IARC 1978; Windholz 1976; National Cancer institute [NCI] 1978). Derivatives of p-Phenylenediamine are important antioxidants in synthetic and natural rubbers, petroleum products, cellulose ethers, and alfalfa meal (IARC 1978; Thirtle 1968).

An occupational exposure limit for p-Phenylenediamine recommended by the National Institute for Occupational Safety and Health (NIOSH) has been adopted by the Occupational Safety and Health Administration. The time-weighted average (TWA) concentration is 0.1 mg/m^3 [skin] for up to a 10-hour workday during a 40-hour workweek (NIOSH 2005).

GENERAL BIOLOGY

Enzyme Effects

p-Phenylenediamine

Appiani et al.(1965) reported that the narcotic effect of pentobarbital in rats was potentiated by pretreatment of the animals with p-Phenylenediamine. Microsomes from rats pretreated with p-Phenylenediamine also metabolized both evipan and strychnine in vitro at lower rates than did microsomes from control animals. According to the researchers, the increased drug sensitivity of individuals exposed to p-Phenylenediamine may have been due to a partial inhibition of hepatic microsomal enzymes.

Geratz et al. (1966) observed inhibition of catalase activity in beef liver exposed in vitro to 10^{-4}M (approximately 20% inhibition) to 10^{-2}M (approximately 90% inhibition) p-Phenylenediamine. Studies with the meta, ortho, and para isomers of phenylenediamine indicated inhibition of catalase activity increases in vitro, with increasing instability of the compound toward oxidation. No inhibition of hepatic catalase activity was noted in mice given injections of the para isomer (dose unspecified). The lack of action in vivo may be due to "rapid degradation" of p-Phenylenediamine in the organism.

Geratz (1969) gave p-Phenylenediamine and other trypsin inhibitors to starved rats by gastric intubation to determine their effect on release of pancreatic enzymes. Secretory

stimulation of the pancreas by 0.01 M p-Phenylenediamine was not significantly different than that of the saline control. The author concluded that there was "no strict parallelism" between pancreas-stimulating activity and trypsin-inhibitory strength.

Kadlubowski (1971) reported studies with mice in which a total dosage of 67.6 mg/kg p-Phenylenediamine was given by intramuscular injection during either a 10- or 20-day period. The activity of various enzymes was measured 48 hours following the last injection. When compared to control animals, mice given p-Phenylenediamine for 10 days had a 33% increase in hepatic catalase activity, a 32 to 36% decrease in hepatic succinic dehydrogenase activity, and a 23 to 32% decrease in hepatic cytochrome oxidase activity; no changes were noted in blood catalase activity or in the blood peroxidase index. In animals exposed to p-Phenylenediamine for 20 days, a 38% increase in hepatic catalase activity was observed as compared to control values; no changes were noted in the hepatic activities of succinic dehydrogenase or cytochrome oxidase, in the activity of blood catalase, or in the blood peroxidase index.

Watanabe et al. (1976) administered a test suspension of p-Phenylenediamine in propylene glycol by intraperitoneal injection to male rats in a dose of 100 µmol/kg (in a volume of 2 ml). Blood activities of aspartate aminotransferase and alanine aminotransferase remained essentially unchanged from control values.

Solano et al. (1988) studied ornithine carboxylase activity in the presence of p-Phenylenediamine. The activity of ornithine decarboxylase was determined by measuring the rate of $^{14}CO_2$ evolved from L-[1-^{14}C] ornithine. Partially purified rat liver ornithine decarboxylase was inhibited by p-Phenylenediamine (1 mM). The relative activity of ornithine decarboxylase in the presence of p-Phenylenediamine was 7.5%.

Mathur et al. (1990) exposed 44 male albino guinea pigs (mean weight = 250 ± 10 g) to p-Phenylenediamine (skin painting, 1% w/v solution). The test substance was administered at a dose volume of 0.1 ml/day for 1, 3, 5, and 7 days. Lipid peroxidation was elevated on days 3, 5, and 7, and significant increases in histamine were observed on days 1, 3, 5, and 7. No significant changes in the glutathione content of the skin were noted. The enzymatic activity of aspartate aminotransferase (AST) increased on day 7 and the enzymatic activity of alanine aminotransferase (ALT) increased on days 5 and 7. Tyrosinase activity increased on day 7. The activities of β-glucuronidase, γ-glutamyl transpeptidase (GGT), and ALT enzymes became elevated after 5 and 7 days of p-Phenylenediamine painting.

Mathur et al. (1992) studied the effect of p-Phenylenediamine on enzyme activity, lipid peroxidation, and the histamine content of guinea pig skin. Thirty male albino guinea pigs (mean weight = 250 ± 10 g) were divided equally into the following 3 groups. Group 1 (animals, clipped free of fur, painted daily with 3% solution (w/v) of p-Phenylenediamine in 25% ethanol [dose volume = 0.1 ml] for 15 and 30 days); Group 2 (animals painted with same concentration of p-Phenylenediamine plus hydrogen peroxide (6.0%, 0.1 ml) daily for 15 and 30 days); and Group 3 (animals treated with vehicle only). The activities of acid and alkaline phosphatases, β-glucuronidase, gamma glutamyl transpeptidase, histidase, and tyrosinase were enhanced following the application of either p-Phenylenediamine or p-Phenylenediamine plus hydrogen peroxide. Additionally, lipid peroxidation and histamine content were markedly elevated following exposure.

Effect on Serum Proteins

p-Phenylenediamine

Rabbits given p-Phenylenediamine in oral doses of 20 mg/kg per day for 12 to 13 days had increased blood concentrations of alpha-, beta-, and gamma-globulin 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 days 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. The authors suggested that changes in the serum protein concentration may be related to alterations in vascular permeability (Mikhlin and Marchenko 1972).

Hematological Effects

p-Phenylenediamine

In a study investigating methemoglobin formation by p-Phenylenediamine, Lin and Wu (1973) reported that 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. Methemoglobin as a percentage of total hemoglobin was 3.7 ± 1.0 at 1 hour, 1.4 ± 0.6 at 4 hours, 3.8 ± 1.4 at 7 hours, and 3.6 ± 1.5 at 10 hours after injection. In vitro determinations of methemoblogin were also made. Rat erythrocytes were isolated and incubated with 10^{-3}M p-Phenylenediamine dissolved in dimethyl sulfoxide.

Methemoglobin as a percent of total hemoglobin was 2.0 ± 1.8 at 1 minute, 1.2 ± 0.5 at 5 minutes, 1.8 ± 0.1 at 10 minutes, 1.8 ± 0.1 at 20 minutes, 2.4 ± 0.7 at 30 minutes, 0.5 ± 0.5 at 60 minutes, 3.9 ± 0.9 at 90 minutes, and 3.9 ± 0.9 at 120 minutes of incubation. No methemoglobin formation was observed in erythrocytes incubated with the dimethyl sulfoxide 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 (Lin and Wu 1973) .

Watanabe et al. (1976) reported a study in which 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). The percentage of methemoglobin formed in the blood was 12.9 ± 4.2 five hours after the injection. Methemoglobin formation was also studied in vitro by incubating 0.1 pmol of rat hemoglobin with 0.5 pmol of p-Phenylenediamine at 37°C for 5 hours. Methemoglobin formation in vitro was 12.8 ± 0.4%, whereas the control methemoglobin concentration was 4.2 ± 1.0%.

Re and D'Aleo (1980) reported that 3 groups of 2 female Beagle dogs each were bled 2 days before dosing and 6 and 24 hours after gastric intubation of aqueous p-Phenylenediamine solutions in doses of 1.0, 3.0, and 10.0 mg/kg. 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.

Loehr and Re (1981) reported that a group of 10 pregnant rats received 40 mg/kg p-Phenylenediamine orally on days 8, 9, and 10 of gestation. 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 hours 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%.

Cardiovascular Effects

p-Phenylenediamine HCl

Administration of p-Phenylenediamine HCl in 0.9% sodium chloride as a slow intravenous perfusion (10 mg/100 g or 20 mg/100 g) or as a rapid intravenous injection (5 mg/100 g) induced an irreversible cardiovascular collapse in rats (Cession-Fossion and Lecomte 1971; Lecomte 1971; Lecomte et al. 1972). The cardiovascular collapse was accompanied by a significant increase in blood catecholamine concentrations (Cession-Fossion and Lecomte 1971), but the cause of the collapse could not be explained by a liberation of endogenous amines from mastocytes or by consumption of kininogens (Lecomte 1971). p-Phenylenediamine HCl (20 mg/100 g) given by intravenous perfusion had no effect on the elevation of blood pressure induced in rats by adrenalin or noradrenalin (Cession-Fossion and Lecomte 1971; Lecomte et al. 1972) and had no direct medullary excitatory action (Lecomte et al. 1972).

Immune System Effects

p-Phenylenediamine

Samter (1970) observed massive peribronchial infiltrates of eosinophils in guinea pigs 72 hours after intrapulmonary injection of an aqueous solution containing 1% p-Phenylenediamine. Antigen-induced infiltrates of eosinophils were limited to the injected lung; no eosinophilia developed in the blood, and no infiltrates of eosinophils were detected in the noninjected lung, which served as the control. The author noted that although anaphylactic sensitivity to p-Phenylenediamine is known, the hair dye intermediate generally induces a delayed sensitivity that is not associated with eosinophilia.

A 0.9% sodium chloride solution containing 100 µg/ml p-Phenylenediamine failed to induce release of histamine or 5-hydroxytryptamine when incorporated into isolated rat mast cells (Lecomte and Baeckeland 1971).

p-Phenylenediamine added to rat peritoneal mast cell cultures at concentrations of 20 to 300 ng/ml had no effect on degranulation (Lecomte et al. 1972).

Using histochemical staining techniques, Shelley and Juhlin (1977) discovered a selective uptake of p-Phenylenediamine by Langerhans cells in isolated guinea pig and human epidermis. It was postulated that Langerhans cells were the site of hapten binding and antigen formation as well as the central target cells in immune contact dermatitis reactions involving p-Phenylenediamine.

Effect on Melanogenesis

p-Phenylenediamine

Lerner and Fitzpatrick (1950) observed that p-Phenylenediamine inhibits melanin formation in vitro. The authors suggested that hair dye intermediate combined with ortho-quinones, thereby preventing the oxidation of dopa-quinone to melanin. Inhibition by p-Phenylenediamine of melanin formation in vivo has not been reported

Brotherton (1969) found that incubation of 10 mM p-Phenylenediamine with cultures of both white and black pig skin 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. This author suggested that p-Phenylenediamine was an inhibitor of tyrosinase, an enzyme that catalyzes the oxidation of tyrosine to dopa and the oxidation of dopa into melanin.

Metabolic Effects

p-Phenylenediamine

O'Neill et al. (1965) reported that a concentration of 10^{-3}M p-Phenylenediamine stimulated the hexose, monophosphate glycolytic pathway in isolated guinea pig brain tissue and inhibited glycolysis by the Embden-Meyerhof-Parnas pathway. Addition of the hair dye intermediate to the cerebral cortex slices as an electron acceptor caused an increase in $^{14}CO_2$ from [1-^{14}C]glucose when compared with that from [6-^{14}C]glucose.

Cilento and Zinner (1967) suggested that, in studies of electron transport in which p-phenylene diamines are used as mediators between the respiratory chain and the substrate, the substrate oxidation may, in part, bypass the respiration-phosphorylation chain. Thus, a lowering of the P:O ratio (molecules of adenosine diphosphate [ADP] phosphorylated:atoms of oxygen reduced) would be expected as a result of increasing concentrations of p-Phenylenediamine.

Gupta et al. (1991) studied the effect of a single concentration of p-Phenylenediamine on glutathione-S-transferase at various time intervals. Groups of 8 female albino strain CDRI guinea pigs (4 test, 4 controls per group; weights = 200 to 250 g) were used. In the first experiment, patches containing 0.1 ml of p-Phenylenediamine (0.9% in 25% ethanol) were applied for 24, 48, or 72 hours. In another experiment, 3 concentrations of p-Phenylenediamine solutions (0.45%, 0.9%, and 1.8%, all in 25% ethanol) were applied and observations were made after 24 hours.

At 24 hours, there was no significant, dose-dependent change in glutathione-s-transferase activity in the postmitochondrial fraction (prepared from guinea pig skin) at either test concentration. The maximum increase in glutathione-S-transferase activity (71% increase) was induced by 0.9% p-Phenylenediamine at 48 and 72 hours.

Microscopic examination of the skin at 24 hours post-application revealed evidence of hyperkeratosis. Significant changes, compared to the control, were not observed in other epidermal layers or in the dermis. However, at 48 hours, discontinuity was observed in areas of the stratum germinativum, indicating a toxic risk to the skin. A moderate degree of edema was observed in the dermis (Gupta et al., 1991).

Mathur et al. (2005) studied the effect of p-Phenylenediamine on the skin using groups of 4 guinea pigs (weights = 250 ± 10 g). In the control group, p-Phenylenediamine (1.0% solution in 25% ethanol) was applied topically to a 2 cm x 2 cm area of skin (clipped free of hair). Another group of guinea pigs was treated with 4 mg/kg p-Phenylenediamine daily for 30 days, and a third group was treated with 4 mg/kg p-Phenylenediamine + 12 mg/kg linear alkylbenzene sulfonate for the same duration. At the end of treatment, control and test animals were killed. Sections of treated skin sites were obtained for biochemical studies and histopathological examination.

Compared to the control, dosing with p-Phenylenediamine caused a significant increase in β-glucuronidase, glutathione-s-transferase, and glutathione peroxidase activities over the 30-day dosing period. p-Phenylenediamine also caused a significant increase in lipid peroxidation and histamine levels. The authors concluded that, compared to control animals, the repeated dermal applicaton of p-Phenylenediamine induced damage to the skin. They also noted the following changes following simultaneous treatment with p-Phenylenediamine and linear alkylbenzene sulfonate: severe hyperkeratosis, vacuolization of epidermal cells, and thickening of collagen fibers (Mathur et al. 2005).

Inhibition of Prostaglandin Biosynthesis

p-Phenylenediamine

Endogenous prostaglandin biosynthesis in homogenates of rat brain was inhibited 58 to 67% by 2 x 10^{-4}M p-Phenylenediamine (Schaefer et al. 1978).

Cytotoxicity

p-Phenylenediamine

Interference with mitosis was observed in intestinal cells of mice given a 0.05 mg intraperitoneal injection of p-Phenylenediamine (Parmentier 1949; Saruta et al. 1958).

Glutathione depletion, lipid peroxidation and cell lysis were observed in isolated rat hepatocytes treated with 1.0 mM p-Phenylenediamine (Anundi et al 1979).

Shigematsu et al. (1988) evaluated the cytotoxicity of p-Phenylenediamine using epidermal cell suspensions from JY-1 guinea pigs (inbred strain). The cells were cultured for 24 hours and p-Phenylenediamine was added at concentrations of 1 to 20 ppm over a period of 48 hours. At a test concentration of 10 ppm, the number of adherent cells was decreased (21% to 40%) when compared to control. At a test concentration of 20 ppm, an even greater decrease (41% to 60%) in the number of adherent cells was reported.

Chung et al. (1996) studied the potency of p-Phenylenediamine in causing cytotoxic effects in Chinese hamster ovary cells. A TC_{50} (50% toxic concentration) of 29 ± 4 ppm was reported.

The results of a study by Picardo et al. (1996) indicated that p-Phenylenediamine induced oxidative stress in normal human keratinocytes in culture. Depending on the p-Phenylenediamine concentration and the period of exposure, peroxidative damage, with a significant decrease in membrane polyunsaturated fatty acids, was detected.

Concentrations between 0.5 and 2 µg/ml produced an initial increase and, then, a decrease in both superoxide dismutase and catalase activities and in the oxidation of reduced glutathione (GSH) for up to 12 hours. After 24 hours (decomposition of p-Phenylenediamine complete), the recovery of initial levels of the antioxidants was detected. Concentrations greater than 5 µg/ml induced a progressive decrease in enzymatic activity and GSH concentrations.

Chen et al. (2006) investigated the mechanism of toxicity of p-Phenylenediamine on the growth of Mardin-Darby canine kidney cells. The cells were grown for 24 hours, and, after 60% confluency was attained, the cells were added to different concentrations of p-Phenylenediamine in DMSO (12.5, 25, 37.5, or 50 µg/ml). DMSO (0.1%) served as the solvent control. Using flow cytometry, a dose-dependent accumulation of the sub-G1 peak and the G0/G1-phase arrested in the cell cycle and a time-dependent induction of apoptosis were observed. Dose-dependent DNA fragmentation (considered biological hallmark of apoptosis), the reduction of membrane potential by mitochondrial membrane depolarization, and an increase in the expression of p53 protein in cells were reported. The evidence of p-Phenylenediamine induced DNA fragmentation into nucleosomes was DNA laddering after 24 hours of incubation. These changes suggested that the effect of p-Phenylenediamine on overall viability and cell numbers was mediated by an increase in apoptosis.

The authors noted that though p-Phenylenediamine-induced apoptosis in Mardin-Darby canine kidney cells was reported in this study, relationships between inhibition of replication, transcription modulation, and apoptosis induced by p-Phenylenediamine remain to be studied. They added that the interaction between apoptosis and carcinogenesis also warrants further investigation (Chen et al., 2006).

p-Phenylenediamine HCl

Cytotoxicity testing of p-Phenylenediamine HCl (in ≤ 0.5% acetone or ≤ 0.5% dimethyl sulfoxide [DMSO]) was conducted in parallel with the testing of this dye in the C3H/10T1/2 cell transformation assay at 2 different laboratories. 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-hour 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 (Dunkel et al., 1988).

Antiparasitic Activity

p-Phenylenediamine HCl

p-Phenylenediamine HCl demonstrated schistosomicidal activity when given orally to mice infected with *Schistosoma mansoni* (Nabih and Helmy 1965). The hair dye intermediate also possessed insecticidal and tuberculostatic properties (Nabih and Helmy 1965; Block et al. 1947).

ABSORPTION, DISTRIBUTION, METABOLISM and EXCRETION

p-Phenylenediamine HCl

Ioannou and Matthews (1985) studied the absorption, distribution, metabolism, and excretion of p-Phenylenediamine HCl using male and female Fischer 344 rats (8 weeks old; weights = 180 to 200 g) and male and female B6C3F_1 mice (6 to 8 weeks old; weights = 18 to 25 g). For administration (i.v. or oral), p-Phenylenediamine was dissolved in a solution of (1:1) ethanol and Emulphor EL-620 (polyoxyethylated castor oil), and water was added to yield a final solvent ratio (ethanol-Emulphor:water) of 1:1:8. Radiolabeled p-Phenylenediamine diluted, as needed, with nonlabeled p-Phenylenediamine was used 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. The i.v. dose of 600 µmol/kg (65 mg/kg) was administered by injection into a tail vein for tissue distribution studies. Each mean value relating to the distribution or excretion of p-Phenylenediamine-derived radioactivity was obtained with 3 animals.

The is study showed that, in rats and mice (both sexes of each species), p-Phenylenediamine HCl was readily absorbed, distributed to all major tissues examined, and metabolized to several metabolites (metabolites observed on high performance liquid chromatography (HPLC) chromatogram not named). These metabolites are rapidly cleared from the body (mainly through the urine), and, to a lesser extent, through the feces. The results of a comparison of p-Phenylenediamine HCl distribution and excretion following administration (oral or i.v.) to rats and mice suggested that gastrointestinal absorption was nearly complete and that excretion was not greatly affected by the route of administration or dose. Furthermore, absorption and excretion were not identical, but were comparable across species and sexes. Absorption was described as rapid, and, in most cases, excretion in urine was more than 90% complete within the first 24 hours.

The authors reported only 2 sex-related differences in the concentrations of p-Phenylenediamine HCl-derived radioactivity in the tissues of mice. Male mice had higher concentrations in the liver, whereas, females had higher concentrations in muscle. When residual concentrations of p-Phenylenediamine HCl-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 (Ioannou and Matthews 1985).

Percutaneous Absorption

p-Phenylenediamine

Maibach and Wolfram (1981) added [C^{14}]p-Phenylenediamine to a commercially available hair dye, and the manufacturer's instructions were used to dye the hair of rhesus monkeys and humans.

Three monkeys were anesthetized, and the dye lotion (2.5 g of the dye solution and 2.5 g of 6% hydrogen peroxide) was worked into their dry scalp hair for approximately 3 minutes and left on for an additional 20 minutes, and then the hair was rinsed, towel-dried, and shaved. Urine was collected from the monkeys at 6, 12, and 24 hours, and then at 24-hour intervals for 7 days. Radioactivity determinations were made on the hair and urine. The 3 monkeys excreted in their urine ranges of 0.083 to 0.190% of the applied radioactivity during the 7 days following hair dye use. Ranges of 12.9 to 14.5% of the applied radioactivity were measured in the hair of the monkeys.

[C^{14}]p-Phenylenediamine was added to a commercially available hair dye, and the manufacturer's instructions were used to dye the hair of humans. The dye mixture (~110 g total, mixture of dye solution and 6% hydrogen peroxide) was applied to the dry hair of 5 humans. It was worked in for 5 to 8 minutes and left on for an additional 20 minutes. The hair was rinsed, towel-dried and shaved. The subjects collected urine for the time periods 0 to 4, 4 to 8, 8 to 12, and 12 to 24 hours, and then at 24-hour time periods up to 144 hours. Radioactivity determinations were made on the hair and urine.

The 5 subjects excreted in their urine ranges of 0.072 to 0.207% of the applied radioactivity during the 7 days following hair dye use. Ranges of 14.1 to 26.5% of the applied radioactivity were measured in the hair (Maibach and Wolfram 1981).

As noted earlier, hair dyes are normally used once every 6 weeks, and maximum exposure to p-Phenylenediamine would occur in users of black oxidation hair dyes. These users would apply approximately 100 g of a dye composition containing up to 2 g of p-Phenylenediamine on each occasion. If approximately 0.2% of the applied p-Phenylenediamine was absorbed percutaneously, a 50 kg person would absorb 80 µg/kg at each hair dye application (CTFA 1982a).

Bronaugh and Congdon (1984) studied the percutaneous absorption of a homologous series of hair dyes (p-Phenylenediamine included) through human epidermis from abdominal skin. 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 area 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 hours, the ratio of the amount of dye in each solvent was determined.

An octanol/water partition coefficient of 0.5 and a permeability constant of 2.4 x 10^{-4} cm/h were reported for p-Phenylenediamine. The authors noted that since other factors, such as binding to proteins in the skin, play a role in determining the absorption rate, oil/water partition coefficients alone provide limited predictive information (Bronaugh and Congdon 1984).

Kim et al. (1987) incorporated [^{14}C]-p-Phenylenediamine (1% in petrolatum) into a variety of patch test systems at a concentration that was normalized to equal a surface area of 2 mg/mm^2. Skin absorption in the guinea pig was determined by evaluating the urinary excretion of ^{14}C. In decreasing order, % skin absorption from the systems were Hill Top chamber (53.4 ± 20.6) > Teflon control patch (48.6 ± 9.3) > small Finn chamber with paper disc insert (34 ± 19.8) > small Finn chamber (29.8 ± 9.0) > large Finn chamber (23.1 ± 7.3) > AL-test chamber (8.0 ± 0.8).

White et al. (2007) conducted a skin binding study (6 male and 6 female Sprague Dawley/Charles River CD [SPF] rats, 6 weeks old) in conjunction with the human study that is summarized in the Clinical Assessment of Safety section (Skin Sensitization subheading) later in the report text. A ^{14}C-p-Phenylenediamine prototype hair dye base vehicle (also containing other intermediates and couplers to mimic in-use conditions) was prepared to yield a test concentration of 3.5 mg p-Phenylenediamine/g base vehicle. p-Phenylenediamine was applied to 10 cm^2 of skin (previously rinsed with detergent solution) in a freshly prepared prototype base formulation mixed with peroxide developer (1:1 by weight). Thus, the amount of p-Phenylenediamine applied was approximately 35 $\mu g/cm^2$. Treatment of the 3 groups of 2 rats was described as follows: Group 1 (single 5-min application; killed at day 1), Group 2 (single 5-min application; killed at day 3), and Group 3 (3 daily 5-min applications; killed at day 3).

The total amounts of penetrated and excreted radioactivity were as follows: Group 1 (0.89 µg equivalents of p-Phenylenediamine), Group 2 (0.92 µg equivalents of p-Phenylenediamine), and Group 3 (5.1 µg equivalents of p-Phenylenediamine). The overall recoveries of the total applied radioactive dose in the 3 groups were: 102.7% (Group 1), 102.6% (Group 2), and 102.6% (Group 3). Cumulative time and single exposure time sites correlated with regard to the retention of radiolabeled test substance in the skin (White et al. 2007).

p-Phenylenediamine HCl

Kiese et al. (1968) applied p-Phenylenediamine HCl in gels and fluids, such as those used in human hair dyeing, to the

skin of dogs, and absorption was calculated from the concentrations of p-Phenylenediamine observed in the blood or the amounts excreted in the urine. Correction factors were determined by observing concentrations in blood and urine after intravenous infusion or subcutaneous injection of known amounts of p-Phenylenediamine HCl. Male dogs were trained to lie on their backs for 3 hours with their legs held loosely after application of p-Phenylenediamine HCl preparations to their abdominal skin. After the 3 hours had elapsed, the dogs were washed with soap and water and were rinsed.

In the first series of experiments, a gel containing 2.5 g of p-Phenylenediamine HCl (1.5 g p-Phenylenediamine) in a mixture of 25 ml of oleic acid, isopropanol, ammonia, higher alcohol sulfates (mainly lauryl sulfate), sodium ethylenediamine tetraacetate, sodium sulfite and perfume, and 25 ml of water or 6% hydrogen peroxide, adjusted to pH 9.5, was used. The gel with water was applied to the skin and covered with aluminum foil or left uncovered and spread from time to time with a spatula. The gel with hydrogen peroxide was applied and left uncovered. p-Phenylenediamine was measured in the blood after application of the gel with water, and it was determined that absorption was favored in the covered application.

The blood concentrations at 3 hours were 0.15 and 0.5 µg/ml for the uncovered and covered gel with water applications, respectively. The gel was washed off the skin after 3 hours, and the p-Phenylenediamine concentrations in the blood slowly dropped over the next 3 hours.

Absorption was stimulated by a continuous intravenous infusion of p-Phenylenediamine HCl into anesthetized dogs. The amounts of p-Phenylenediamine that must have been absorbed to result in various blood concentrations of p-Phenylenediamine were calculated. The intravenous infusion with constant velocity did not perfectly simulate the absorption of p-Phenylenediamine through the skin. It was calculated that 11 mg/kg of p-Phenylenediamine was absorbed when the gel with water was applied under cover to the skin. In the gel with water application without cover, a total of 16 mg/kg of p-Phenylenediamine was absorbed. No p-Phenylenediamine was found in the blood when gel with H_20_2 was used for skin application. Since intravenous infusions of 0.001 mg/kg per minute of p-Phenylenediamine yielded detectable blood concentrations, the absorption must have been less than 2 mg.

In a second series of experiments, a fluid containing 0.6% p-Phenylenediamine in 30 ml of detergent, a phosphoric acid ester of a higher alcohol, sodium hydroxide, and perfume, 10 ml of isopropanol, and 10 ml of water, adjusted to pH 9.5, was applied to the skin of dogs. p-Phenylenediamine absorption was estimated by determining the amount excreted in the urine after it was injected subcutaneously. Following subcutaneous injection at doses of 0.6, 1.2, and 3 mg/kg, the average amounts of p-Phenylenediamine found in the urine of dogs (as calculated from 3 experiments) with each dose were proportional to the dose injected. Even though the concentration of p-Phenylenediamine in the fluid was lower than in the gel, the total amount of p-Phenylenediamine absorbed was approximately the same from the fluid as from the uncovered gel with water application. Absorption was less if resorcinol and 2,4-diaminophenol were added to the fluid preparation. Bandrowski's base was absorbed through the skin and was detected in the urine, although not in the blood (Kiese et al. 1968).

Rehani et al. (1981) applied ~ 3 µg of [H^3]p-Phenylenediamine HCl to 2 shaved areas on mice, and the applications were repeated 20 minutes later. Mice were killed over a 3-day period, and tissue (same tissues as after intravenous administration) and blood samples were taken. The percutaneous absorption of radioactivity was rapid. Despite its rapid clearance from the blood (concluded from the previous experiment), there was a steady rise in concentration of radioactivity in blood over the first 24 hours. The maximum concentrations of radioactivity at 3.5 hours were found in the brain, at 24 hours in the brain, liver, and stomach, and on the second day in the liver, stomach, and adrenals.

Steiling et al. (2001) studied the percutaneous absorption of p-[U-^{14}C]Phenylenediamine HCl using intact, full-thickness skin from young domestic pigs (12 skin samples total) in glass diffusion cells. Skin discs were mounted between the receptor chamber (filled with receptor solution) and the donor chamber. The dermis was in close contact with the receptor solution. p-Phenylenediamine HCl (formulated in standard cream) was applied without occlusion in a total amount of 20 mg/cm^2. Exposure to the topically applied dye was terminated after 30 minutes by rinsing with mild shampoo and deionized water. The receptor fluid was continuously pumped through the receptor chamber and sampled over a 24-hour period. At study termination, skin samples were tape-stripped in order to remove the horny layer. Residual skin samples, after taking approximately 20 tape strips, were analyzed for the amount absorbed. Most of the p-Phenylenediamine (85 to 89%) was found in the washing solutions. Adsorbed *p*-Phenylenediamine in the tape (~3%) was also reported. The percutaneous penetration rate for p-Phenylenediamine was 0.3%. The absorbed quantity of p-Phenylenediamne was limited to 0.6% in the presence of hydrogen peroxide.

Hueber-Becker et al. (2004) investigated the absorption of a commerical p-Phenylenediamine HCl-containing oxidative dark-shade in 8 human volunteers as well as in vitro using human or pig ear skin. For the in vivo study, the isotopic dilution of [^{14}C]p-Phenylenediamine was prepared by

mixing 1.6 Mbq [^{14}C]p-Phenylenediamine Dichloride with 40 ml of a commerical dark-shade oxidative hair dye formulation containing 3.98% cold [^{14}C]-p-Phenylenediamine HCl and 2.0% meta-aminophenol. This resulted in an isotopic dilution to a specific activity of 54.7 disintegrations per mimute (DPM) per μg_{equiv}. The actual content of [^{14}C]p-Phenylenediamine HCl in the hair dye formulation after the isotopic dilution and prior to mixing with the developer was determined to be approximately 4%. The same commercial products were used for the in vitro study.

The hair of each subject was dyed, washed, and then collected. Hair, washing water, materials used in the study, and a 24-hour scalp wash were obtained for determination of radioactivity. Blood, urine, and feces were analyzed for up to 120 hours after dyeing of the hair. An identical [^{14}C]p-Phenylenediamine HCl-containing hair dye formulation was applied for 0.5 hour to both human and pig ear skin *in vitro*; radioactivity was determined in skin compartments after 24 hours.

For humans, the recovery rate was 95.7 ± 1.5% of the applied radioactivity. Washing water, cut hair, gloves, paper towels, caps or scalp wash contained a total of 95.16 ± 1.46% of the applied [^{14}C]. Absorbed radioactivity amounted to 0.50 ± 0.24% in the urine and 0.04 ± 0.04% in the feces. This corresponded to a mean of 7.0 ± 3.4 mg [^{14}C]-p-Phenylenediamine HCl-equivalents absorbed. Most of the radioactivity was eliminated within 24 hours after application. The peak concentration (C_{max}) of [^{14}C]-p-Phenylenediamine HCl-equivalents in the plasma was 0.087 μg_{eq}/ml, and the time-to-peak concentration (T_{max}) was approximately 2 hours; the mean area under the curve ($AUC_{0\text{-}12\ hours}$) was 0.67 μg_{eq} h/ml.

The results of *in vitro* tests using human or pig skin revealed total absorbed amounts of 2.4 ± 1.6% (10.6 ± 6.7 $\mu g_{eq}/cm^2$) or 3.4 ± 1.7% (14.6 ± 6.9 $\mu g_{eq}/cm^2$), respectively. Percentage-based in vitro results were considerably higher than corresponding in vivo data. However, in units of $\mu g/cm^2$, they corresponded to a total absorbed amount of 7.40 or 10.22 mg_{eq} for human or pig skin, respectively.

The authors concluded that the results of this study suggested that dyeing the hair with oxidative dyes produces minimal systemic exposure that is unlikely to pose a risk to human health (Hueber-Becker et al. 2004).

Distribution

p-Phenylenediamine HCl

Rani et al. (1979) administered [H^3]p-Phenylenediamine HCl to rabbits by subconjunctival injection, intravitreal injection, local drops, and subcutaneous injection into the head. The aqueous fluid from the anterior chamber of the eye was examined for radioactivity. There was rapid clearance from the site of administration. Detectable amounts of radioactivity were found in the aqueous fluid 15 to 30 minutes after administration. The peak concentration was reached within a half-hour for subconjunctival injection and local drops and within 1 hour for subcutaneous and intravitreal injection. With the exception of application by subcutaneous injection, the concentration of radioactivity in the aqueous chamber fluid was less than 5% of the peak concentration after 4 days. Thirty percent of the peak concentration of radioactivity in the aqueous chamber fluid was found 4 days after subcutaneous injection. The half-life of clearance of p-Phenylenediamine from the aqueous chamber fluid after subcutaneous injection was 3.8 ± 0.5 days.

Rehani et al. (1981) administered ~ 15 µg of [H^3]p-Phenylenediamine HCl diluted in normal saline intravenously to a rabbit. Blood was drawn over an 8-hour period, clearance of p-Phenylenediamine from the blood was fitted to a biexponential curve, and the curve indicated a biphasic clearance of the radioactivity from the blood with half-life values of 24 minutes and 43.5 hours. In another experiment, approximately 30 pg was applied to a shaved area on the abdomen of a rabbit. After 20 minutes, only 0.05% of the applied radioactivity could be detected in the blood.

The tissue distribution of radioactivity was studied after intravenous administration of labeled p-Phenylenediamine to mice. p-Phenylenediamine HCl (^{3}H) was diluted with saline and approximately 3 pg was administered intravenously. Mice were killed over a 12-day period, and tissue (liver, heart, kidney, stomach, intestine, adrenals, testes, muscle, bone marrow, bone, brain, skin, thyroid, eyes, spleen, and lungs) and blood samples were taken. Greater than 85% of the radioactivity was cleared from the blood within the first day. No target organ was apparent. The maximum concentration of radioactivity was located in the stomach and kidney at 1 hour, in the skin at 1 day, in the spleen at 2 days, in the muscle at 3 days, and in the bone marrow at 5 days. On the seventh day, less than 0.5% of the injected radioactivity was retained in any of the tissues examined (Rehani et al. 1981).

p-Phenylenediamine and p-Phenylenediamine HCl

Marzulli et al. (1978) applied [C^{14}]p-Phenylenediamine (as the free base) at a dose of 4 pg/cm^2 to the forearm of 6 human subjects. Within 5 days 12.7 ± 6.98% of the radioactivity was recovered in the urine.

[C^{14}]p-Phenylenediamine was applied as the HCl salt to the same subjects, and 14.8 ± 5.5% of the radioactivity was recovered in the urine. Metabolic transformation was not evaluated in this study.

The authors stated that these values would be expected to be higher if they were corrected by adjusting for amounts found

in urine after intravenous injection of known amounts of p-Phenylenediamine (Marzulli et al. 1978).

Nakao and Takeda (1979) administered [C^{14}]p-Phenylenediamine intraperitoneally in doses of 1.5 mg/kg to male rats, and the radioactivity was followed over time. The highest concentration of radioactivity was found 1 hour after administration in tissues other than the intestines, and this was followed by a rapid decrease in concentration. Large amounts of radioactivity were found in the intestinal tract and in the urine. Only very small amounts of radioactivity were found in the tissues after 48 hours. Sixty-nine percent of the administered radioactivity was excreted in the urine, 29% was excreted in the feces, and 26% was in the bile within 24 hours. The male rats rapidly excreted p-Phenylenediamine after its intraperitoneal administration. N,N'-diacetyl-p-Phenylenediamine, p-aminoacetanilide, and unchanged p-Phenylenediamine were identified as urinary metabolites. Thirty percent of the radioactivity in the urine was accounted for by N,N'-diacetyl-p-Phenylenediamine.

Metabolism

p-Phenylenediamine

Goetz et al. (1988) conducted a study using 5 female volunteers who were long-time users of oxidative hair dyeing products. They determined that, during an on-line flash hydrolysis of their urine, several metabolites of p-Phenylenediamine were hydrolyzed to free p-Phenylenediamine. The authors monitored the excretion of metabolites during 24 or 48 hours after application of the dye. The major metabolite that was determined using this approach was N,N'-diacetyl-p-Phenylenediamine (DAPPD). Approximately 80% of the p-Phenylenediamine recovered after flash hydrolysis was from the hydrolysis of N,N'-diacetyl-p-Phenylenediamine.

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 hours was recovered during the first 24 hours (Goetz et al., 1988).

In a study by Wang and Tsai (2003), p-Phenylenediamine (60 mg, with 10 ml of water) was administered orally to male and female rabbits (weights = 2836 to 4299 g). Following administration, p-Phenylenediamine and its metabolites were measured in the serum. Serum concentrations varied from 0.213 to 0.018 µg/ml for p-Phenylenediamine, 0.111 to 0.030 µg/ml for N-acetyl-p-Phenylenediamine, and 3.02 to 0.85 µg/ml for N,N'-diacetyl-p-Phenylenediamine. Levels of metabolites peaked during the first 0.5-hour collection interval.

Maximum absorption of p-Phenylenediamine (2.20 µg/ml) occurred at 1.5 hours 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 N,N'-diacetyl-p-Phenylenediamine and the minor metabolite was N-acetyl-p-Phenylenediamine.

These authors also examined urine samples from 5 volunteers (20 to 24 years old) who had used a commercial hair dye containing 1.1 to 1.6 g of p-Phenylenediamine. The major metabolite was DAPPD. This diacetyl derivative of p-Phenylenediamine was excreted in the urine for 42 hours after hair dyeing. The average amount was 0.14 µg/ml per person (Wang and Tsai 2003).

In a study by CIT Safety & Health Research Laboratories (2005a), the plasma pharmacokinetics and mass balance (over 24 hours) of total radiaoactivity were investigated following single oral gavage administration of ^{14}C-p-Phenylenediamine at 6.45 mg/kg to male and female Sprague-Dawley rats. Additonally, the metabolic profile in plasma was investigated after dermal application (4-hour exposure, under occlusive conditions) at a dose of 49.9 mg/kg. Following single oral gavage, the plasma radioactivity versus time profiles showed a fast absorption phase (t_{max} = 0.5 hour) with a C_{max} of 7115/6880 ng-eq/g for males/females. Overall mean exposure (AUC_{0-t}) for males/females was 24847/27304 ng-eq/g*h. The mean minimal fraction (radioactivity found in urine) absorbed was high, 74.4/81.0% of the dose for males/females in the 24-hour period. Recovery was complete at 103.8/104.4%, with 19.3/13.8% in the feces, 3.1/4.8% in the cage wash, and 7.0/4.7% in the carcasses.

Following single dermal application, 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 the diacetylated p-Phenylenediamine only. All of the data were characterized by a low inter-animal variability. However, there were generally no gender differences (CIT Safety & Health Research Laboratories 2005a).

In a study by CIT Safety & Health Research Laboratories (2005b), the plasma pharmacokinetics and mass balance (over 24 hours) of total radioactivity were investigated following a single oral administration of ^{14}C-p-Phenylenediamine (dose = 4 mg/kg) to male and female Sprague-Dawley rats. Following oral gavage, the plasma radioactivity versus time profiles showed a fast absorption phase (t_{max} of 0.5 hour), with a C_{max} of 4098 and 3729 ng-eq/g for males and females, respectively. Overall mean exposures (AUC_{0-t}) for males and females were 10,842 and 10,797 ng-eq/g*h, respectively. The mean minimal fraction (radioactivity found in urine) that was absorbed was high, 57.0 and 60.1% of the dose for males and females, respectively (in the 24-hour period). Recovery was complete at 91.8 and 92.0% in the 24-hour period, with 23.7

and 19.3% in the feces, 7.3 and 8.3% in the cage wash, and 3.7 and 4.2% in the carcasses (each set of values for males and females, respectively). All of the data were characterized by a low inter-animal variability. There were no gender differences and there was no evidence of enterohepatic recycling (CIT Safety & Health Research Laboratories 2005b).

In Vitro Metabolism

p-Phenylenediamine

Stanley et al. (2005) used intact human hepatocytes, human liver microsomes, and heterologously expressed human cytochrome P450s (CYPs) to determine whether [^{14}C]-p-Phenylenediamine is metabolized by hepatic CYPs to form an N-hydroxylamine. 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.

The authors noted that the absence of evidence for hepatic CYP-mediated metabolism of [^{14}C]-p-Phenylenediamine is inconsistent with the hypothesis that this chemical plays a causal role in the development of bladder cancer via a mode of action that involves hepatic metabolism to an N-hydroxyarylamine (Stanley et al. 2005).

Dermal Metabolism

p-Phenylenediamine

Nohynek et al. (2005) investigated the biotransformation of the oxidative arylamine hair dye ingredients [^{14}C]-p-aminophenol (PAP) and [^{14}C]-p-Phenylenediamine in reconstructed human epidermis and human hepatocytes. In human epidermis, PAP was quantitatively transformed into its N-acetylated derivative (APAP). However, in human hepatocytes, PAP was transformed into sulfate or glucuronic acid conjugates of APAP or PAP, as well as free APAP. In human hepatocytes and in the human epidermis, [^{14}C]-p-Phenylenediamine was converted to MAPPD and DAPPD derivatives. At higher concentrations of [^{14}C]-p-Phenylenediamine (250 to 1000 µM), the epidermis or 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 or DAPPD was 3-fold or 8-fold greater, respectively. There was no evidence that either PAP or [^{14}C]-p-Phenylenediamine was transformed to N-hydroxylated derivatives in the epidermis or hepatocytes.

The authors stated that the results of this study suggestthat after dermal absorption of PAP or [^{14}C]-p-Phenylenediamine, humans are systematically exposed to acetylated derivatives; current in vitro skin absorption studies may be inadequate for determination of human systemic exposure to arylamines due to reduced or absent metabolic capacity of non-viable skin; due to qualitative differences between dermal and hepatic metabolism, oral toxicity studies may be unsuited for the hazard assessment of dermal exposure to arylamines; and use of induced rodent liver S9 metabolic activation systems for in vitro genotoxicity studies may produce misleading results on the hazard of human dermal exposure to arylamines.

The authors concluded that these data support the growing evidence that arylamines are transformed in human skin and suggest that current practices of safety assessment of arylamines should take these findings into account (Nohynek et al., 2005).

Dressler and Appelqvist (2006) studied the pharmacokinetics and metabolism of [^{14}C]-p-phenylenediamine following dermal application using six (3 males, 3 females) 7-week-old female Crl CD® (SD) IGS BR COBS-VAF® rats (3 males, 3 females; mean body weights = 243 g and 164 g, respectively). The test substance was applied to clipped skin in the back and shoulder regions. A volume corresponding to 6 mg of the test solution (50 mg/kg) was applied with a plastic syringe and then spread evenly with a spatula over the exposed skin. During exposure, the application site was covered with an occlusive dressing and protected from the light. At 4 hours post-dosing, an analysis of the plasma indicated levels of 1.41 ± 0.34 µg/ml [^{14}C]-PPD-equivalents in males, and 7.40 ± 1.83 µg/ml in females. Radioactivity revealed a single metabolite, N,N'-diacetylated [^{14}C]-p-phenylenediamine. The results of this study suggest that topically applied [^{14}C]-p-Phenylenediamine is metabolized in the skin, presumably by N-acetyltransferase-1.

Immune Response

p-Phenylenediamine

Sieben et al. (2002) analyzed the recognition and processing requirements of p-Phenylenediamine and its autoxidation product, BB, using peripheral blood mononuclear cells (PBMCs) from human subjects. The subjects were allergic to p-Phenylenediamine and p-Phenylenediamine- and BB-reactive T cells. Study results suggest that p-Phenylenediamine itself can be recognized by T cells through a processing-independent pathway, whereas its autoxidation product (BB) required processing and, possibly, metabolism to stimulate the same T-cell clones. These data demonstrate that two distinct pathways of antigen presentation to activate specific T-cell clones are involved

in the immune response to p-Phenylenediamine.

Yokozeki et al. (2003) contact-sensitized mast cell-deficient WBB6F-W/W^v female mice and their congenic normal (+/+) mice by following a procedure of daily topical applications of 50 µl of 2.5% p-Phenylenediamine solution in acetone, in olive oil (1:4), on shaved abdominal skin. C57BL/6 mice and STAT6$^{-/-}$ mice were contact sensitized by 5 daily consecutive topical applications of 50 µl of 2.5% p-Phenylenediamine with 3% H_2O_2. At 2 days after the last abdominal application, the mice were challenged by applying 20 µl of 2.5% p-Phenylenediamine solution on both sides of one ear and vehicle on both sides of the other ear. The results of this study showed that T helper 2 (Th2) cytokines, immunoglobulin E (IgE), and mast cells play an essential role in the induction of p-Phenylenediamine-induced hypersensitivity in mice.

Cruz et al. (2005) used flow cytometry to study the effect of p-Phenylenediamine and other sensitizers on the surface expression of the chemokine receptors CCR6 and CXR4 using a fetal mouse dendritic cell line. This cell line (has morphological, phenotypic, and functional characteristics of skin dendritic cells) served as an experimental model of a dendritic cell. Cells were cultured with 2 concentrations of p-Phenylenediamine (10 and 50 µg/ml). p-Phenylenediamine and the other sensitizers decreased the membrane expression of the chemokine receptors CCR6 and CXCR4 in the fetal mouse dendritic cell line. These receptors regulate the recruitment of antigen-presenting and immunocompetent cells during inflammatory and immunological responses, namely allergic contact dermatitis. The authors noted that the results of this *in vitro* study may be related to the *in vivo* enhancement of dendritic cell migration out of the skin and to the lymph nodes for antigen presentation to the T cells.

Coulter et al. (2007) studied the relationship between p-Phenylenediamine oxidation and the functional maturation of human monocyte-derived dendritic cells *in vitro*. Dendritic cells were incubated with p-Phenylenediamine for 16 hours, and the expression of the co-stimulatory receptors CD40, CD80, CD83, and CD86, major histocompatibility complex class II molecules, intracellular glutathione levels, and cell viability were measured. The proliferation of allogeneic lymphocytes was determined by the incorporation of [^{3}H]thymidine. The exposure of dendritic cells to p-Phenylenediamine (5 to 50 µM) was associated with an increase in CD40 and major histocompatibility complex (MHC) class II expression and proliferation of allogeneic lymphocytes. Dendritric cell activation with p-Phenylenediamine was not associated with apoptotic or necrotic cell death.

ANIMAL TOXICOLOGY

Acute Oral Toxicity

p-Phenylenediamine and p-Phenylenediamine HCl

The acute oral toxicities of p-Phenylenediamine and p-Phenylenediamine HCL and hair dye formulations containing p-Phenylenediamine have been studied in rats (Burnett et al. 1977; CTFA 1969a; Lloyd et al. 1977), rabbits, cats (Hanzlik 1923), and dogs (CTFA 1980). Doses, number of animals, and results are summarized in Table 6.

Acute Dermal Toxicity

p-Phenylenediamine

The acute dermal toxicity of p-Phenylenediamine and p-Phenylenediamine-containing products to rabbits has been studied. The dry, basic form of p-Phenylenediamine and a 10% alcoholic solution of p-Phenylenediamine applied to an approximately 25 cm^2 area of shaved, washed, and dried skin of 3 rabbits resulted in no demonstrable signs of systemic toxicity (Hutzinger 1969).

CTFA (1969b) reported a study in which a hair dye composite formulation containing 1.2% p-Phenylenediamine was applied at a dose of 10 g/kg to the skin on the backs of 2 male and 2 female rabbits. The hair on the backs of the rabbits had been clipped, and the application sites were approximately 10% of the body surface. After application of the composite, the exposure site was wrapped with impervious plastic sheeting for 24 hours. The animals were observed for 14 days following the treatment. The percutaneous LD_{50} of the formulation was greater than 10 g/kg.

Lecomte et al. (1972) observed edema and focal necrosis in rats following skin application of 1 to 5 mg p-Phenylenediamine.

Acute Subcutaneous Toxicity

p-Phenylenediamine HCl

Hanzlik (1923) reported that the minimum fatal doses of p-Phenylenediamine base and hydrochloride in water administered subcutaneously to 10 rats and 3 rabbits were 170 mg/kg and 200 mg/kg, respectively. Edema of the head and neck was observed in some of the rabbits.

In another study, the subcutaneous administration of 350 mg of p-Phenylenediamine HCl to guinea pigs and doses of 120 to 150 mg to rats resulted in edema of the head and neck (Tainter et al. 1929).

Lecomte and Cession-Fossion (1971) reported necrotic lesions and edema at the site of injection following subcutaneous administration of 1, 2, or 5% p-Phenylenediamine HCl in 0.9% sodium chloride. The edema was attributed to an increase in vascular permeability and was accompanied by the release of histamine and 5-hydroxytryptamine from tissues.

Acute Parenteral Toxicity

p-Phenylenediamine

The rat acute intraperitoneal LD_{50} of an aqueous p-Phenylenediamine solution was 37 mg/kg (Burnett et al. 1977)

p-Phenylenediamine HCl

The minimum fatal dose of p-Phenylenediamine base and hydrochloride administered intravenously to 3 dogs was 170 mg/kg (Hanzlik 1923).

The intraperitoneal administration of 190 mg/kg of p-Phenylenediamine HCl to rats and 120 mg/kg to cats resulted in edema of the head and neck (Tainter and Hanzlik 1924).

Short-Term Oral Toxicity

p-Phenylenediamine

Mikhlin and Marchenko (1972) gave p-Phenylenediamine orally at doses of 20 mg/kg per day for 12 to 13 days to

Table 6. Acute Oral Toxicity of p-Phenylenediamine and p-Phenylenediamine HCl.

Material Tested	Dose	Animals	LD_{50}	Comments	Reference
p-Phenylenediamine as base and HCl salt in water	0.1-0.45 g/kg	7 rabbits	-	Minimum fatal dose was 0.170 g/kg. Increase in pulse and respiration and decrease in temperature. Facial, tongue, and neck edema and dyspnea observed in 4 rabbits receiving from 0.2 to 0.22 g/kg. These 4 rabbits eventually died, although there was recovery from the edema and other symptoms. High-dose rabbits died before edema developed	Hanzlik 1923
p-Phenylenediamine as base and HCl salt in water	0.1 g/kg	1 cat	-	Increase in pulse and respiration and decrease in temperature	Hanzlik 1923
Hair dye composite containing 1.156% p-Phenylenediamine	-	2 male and 2 female rats at each dose	41.3 ml/kg for male rats and 38.3 ml/kg for female rats	-	CTFA 1969a
p-Phenylenediamine in oil-in-water emulsion	-	10 rats at each dose	80 mg/kg	-	Burnett et al. 1977
p-Phenylenediamine in water adjusted to pH 7.0	-	2-10 rats at each dose	98 mg/kg	-	Lloyd et al. 1977
p-Phenylenediamine in water	1.0, 3.0, and 10.0 mg/kg	2 female beagles at each dose	-	The 10.0 mg/kg dose dogs showed lacrimation at 2 hours and redness and swelling of conjunctiva at 3 to 6 hours. The eyes were slightly cleared at 24 hours. At 3 hours, the 3.0 mg/kg dose dogs showed slight redness and lacrimation and the 1.0 mg/kg dose dogs showed lacrimation. The lower-dose groups were normal at 24 hours	CTFA 1980
p-Phenylenediamine in water	10.0 mg/kg	2 female beagles	-	At 2 to 6 hours, the dogs developed red and swollen conjunctiva and ocular discharge. At 24 hours the dogs had half-closed eyes and red swollen conjunctiva	CTFA 1980

rabbits. Animals had 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 days 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.

The authors suggested that changes in the serum protein concentration may be related to alterations in vascular permeability (Mikhlin and Marchenko 1972).

CTFA (1981) reported a study in which an aqueous solution of p-Phenylenediamine was administered orally in a dose of 40 mg/kg to 10 pregnant rats on Days 8, 9, and 10 of gestation and in a dose of 30 mg/kg to 10 pregnant rats on Days 6 through 15 of gestation. There were 20 control rats. A decrease in the body weights of the rats given 40 mg/kg p-Phenylenediamine was observed on Days 9 and 10. Two rats given 40 mg/kg died after the third dose. No other differences were observed between control and treated rats.

p-Phenylenediamine HCl

The National Cancer Institute (NCI) studied the short-term oral toxicity of p-Phenylenediamine HCl using rats and mice (NCI 1978). p-Phenylenediamine HCl was fed to rats and mice at dietary concentrations ranging from 68 to 3160 ppm and 100 to 4640 ppm, respectively, for 7 weeks, and the animals were observed for another week. There were 10 animals at each of 11 doses and there were 10 controls. All of the animals survived and no signs of toxicity were observed.

Short-Term Dermal Toxicity

p-Phenylenediamine

In order to determine the effect of superoxide dismutase (SOD) and the combined effect of SOD and p-Phenylenediamine on female guinea pig skin, Viswanathan et al. (1986) applied a patch containing a 0.19 ml solution (50 units of enzyme) and a patch containing a mixture [0.1 ml of 0.9% p-Phenylenediamine and 0.9 ml of solution containing 50 units of enzyme] to the clipped skin of female guinea pigs (weights = 200 to 300 g). Patches containing p-Phenylenediamine (0.1 ml) were applied to clipped ventral and dorsal skin surfaces. The patches were covered with aluminum foil and remained in place for up to 48 hours, after which the animals were killed and the exposed portion of the skin removed and homogenized. Homogenates were used in an assay for lipid peroxidation.

Lipid peroxidation in the ventral and dorsal sides of the skin was reported at 24, 48, and 72 hours. Skin damage due to p-Phenylenediamine on the ventral side was described as 96.7% at 24 hours, 7.18% at 48 hours, and 59.76% at 72 hours. Changes on the dorsal side due to application of the dye on the ventral side were described as 65.98% at 24 hours, 45.83% at 48 hours, and 7.68% at 72 hours. At 24 hours post-exposure, no change in the activity of SOD was noted. The histamine content in the skin of the animals treated with p-Phenylenediamine at different time intervals showed an increase up to the first 48 hours (56.87%) and then decreased up to 96 hours (98%).

Microscopic examination of the skin at 24 h post-application of p-Phenylenediamine revealed evidence of hyperkeratosis, but no significant changes in other epidermal layers or the dermis. At 48 hours, discontinuity appeared in the stratum germinativum and a moderate degree of edema was observed in the dermis.

For SOD-exposed animals, the following changes were noted at 24 hours post-treatment: irregular stratum germinativum, slight edema in the dermis (edema more prominent at 48 hours). Following exposure to p-Phenylenediamine + SOD, there was hardly any indication of abnormal findings in the various layers of the epidermis or dermis up to 48 hours (Viswanathan et al., 1986).

Mathur et al. (1990) exposed 44 male albino guinea pigs (mean weight = 250 ± 10 g) to p-Phenylenediamine (skin painting,1% w/v solution). The test substance was administered at a dose of 0.1 ml/day for 1, 3, 5, and 7 days. The effect of p-Phenylenediamine on lipid peroxidation and several enzymes in the skin and serum were studied. The liver, kidney, and skin were also examined microscopically.

Lipid peroxidation was increased on days 3, 5, and 7. The enzymatic activity of aspartate aminotransferase (AST) increased on day 7 and the enzymatic activity of alanine aminotransferase (ALT) increased on days 5 and 7. Tyrosinase activity increased on day 7. The activities of β-glucuronidase, γ-glutamyl transpeptidase (GGT), and ALT enzymes increased after 5 and 7 days of p-Phenylenediamine painting.

Other than a few focal and early degenerative changes in hepatocytes (along with mild fatty changes), no signs of hepatotoxicity were observed on days 1 and 3. Moderate congestion of the lobules (congestion of sinusoids in particular) was noted by day 5. By day 7, a focal granulomatous reaction, with occasional Langerhans-type giant cells, was noted. No significant histological changes in the cortex or medulla of the kidney were noted at days 1, 3,

5, or 7 post-application.

On day 1, moderate changes in the skin, which comprised hyperkeratosis of the stratum corneum and focal infiltration of polymorphs and mononuclear leukocytes, were observed in the dermis. On day 7, exposure resulted in marked hyperkeratosis together with the infiltration of cells in the dermis; edema and congestion in the dermis persisted. The biochemical defects and microscopic changes in the skin and serum correlated with the duration of exposure (Mathur et al., 1990).

Subchronic Oral Toxicity

p-Phenylenediamine

Imaida et al. (1983) studied the subchronic oral toxicity of p-Phenylenediamine using 5 groups of F344 rats (males and females, 10 to 11 rats per group). Four groups were fed a diet containing 0.4, 0.2, 0.1, or 0.05% p-Phenylenediamine. Group 5 (diet only) served as the control. The animals were killed after 12 weeks. Dose-dependent growth retardation was observed in males and females, especially in the group fed 0.4% p-Phenylenediamine in the diet. Liver-to-body weight and kidney-to-body weight ratios (expressed as %) in the group fed 0.4% p-Phenylenediamine were higher when compared to the control group. In this group, 9 male rats and 1 female rat died before the end of the experiment.

Subchronic Dermal Toxicity

A hair dye composite formulation containing 1.2% p-Phenylenediamine was applied topically to 10 rabbits (1 g/kg dose) 5 times per week for a total of 65 applications. Mild to marked acanthosis and mild to moderate dermal fibroplasia were observed in 7 rabbits (CTFA 1989c). In another study (groups of 12 rabbits), hair dye composite formulations containing up to 4% p-Phenylenediamine were mixed 1:1 with hydrogen peroxide and applied topically 2 times a week in a dose of 1 mg/kg for 13 weeks. No toxic signs were observed (Burnett et al. 1976). Results are shown in Table 7.

Chronic Oral Toxicity

p-Phenylenediamine HCl

The chronic oral toxicity of p-Phenylenediamine HCl has been studied in rats and in mice by the NCI (NCI 1978). p-Phenylenediamine HCl was administered in the feed at concentrations of 625 and 1250 ppm to groups of 100 rats and groups of 100 mice for 103 weeks. The rats were observed for 2 weeks, and the mice for 1 week, following the feeding experiment. Forty animals of each species were controls. No body weight depression or other signs of toxicity were observed in the treated animals.

Chronic Dermal Toxicity

p-Phenylenediamine

Few adverse effects were reported as a result of chronic dermal toxicity studies of p-Phenylenediamine and hair dye formulations containing p-Phenylenediamine in mice (Burnett et al. 1975; Burnett et al. 1980; Giles et al. 1976; Stenback et al. 1977), rabbits (Stenback et al. 1977), and rats (International Research and Development Corporation [IRDC] 1979) as shown in Table 7.

Neurotoxicity

p-Phenylenediamine

In a study by E. I. du Pont de Nemours & Company (1990), groups of 24, 63-day-old Crl:CD® rats (12 males, 12 females; mean body weights = 267.5 ± 1.5 g [males] and 211.2 ± 1.7 g [females]) were dosed orally (by gavage) with p-Phenylenediamine in sterile water at single doses of 20, 40, and 80 mg/kg. 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 (FOB) 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. The authors concluded that p-Phenylenediamine did not exert a primary effect on the nervous system (E. I. du Pont de Nemours & Company 1990).

In another study by E. I. du Pont de Nemours & Company (1992), male and female Crl:CD®BR rats (10 rats/sex/group; weights = 58.8 ± 4.50 g [males] and 59.0 ± 7.73 g [females]) received oral doses of p-Phenylenediamine (in sterile water; 4, 8, or 16 mg/kg doses by gavage) for a minimum of 90 consecutive days. As above, a neurobehavioral test battery consisting of motor activity (MA) and FOB assessments were conducted during the week prior to the onset of the dosing period. This neurobehavioral test battery was conducted again during weeks 4, 8, and 13 of dosing. Following completion of the dosing period, surviving rats were killed and perfused, and neuropathologic evaluations were conducted. The rats were subjected to *in situ* whole body perfusion with heparinized saline, followed by a solution of 10% formalin, 2.5% glutaraldehyde, and 0.5% picric acid with phosphate buffer.

Mortalities related to test substance administration were not observed. Changes in body weights and body weight gains, as well as absolute feed consumption and feed efficiency values, were not treatment-related. There were no test substance-related effects on any of the FOB (e.g., forelimb grip strength, hindlimb grip strength, foot splay) or MA

parameters that were evaluated. Neuropathologic evaluations did not reveal abnormalities in the nervous system or skeletal muscle. There were no effects of the test substance on ocular tissue (E. I. du Pont de Nemours & Company (1992).

Hepatotoxicity

p-Phenylenediamine

No hepatic toxicity was observed in male rats given a single intraperitoneal injection of p-Phenylenediamine in propylene glycol at a dose of 100 pmol/kg (in a volume of 2 ml) (Watanabe et al. 1976).

Myotoxicity

p-Phenylenediamine

Rabbits that received p-Phenylenediamine at oral doses of 20 mg/kg for 12 to 13 days and 10 mg/kg for 90 days had marked alterations in myocardial parenchyma. These changes included edema, swelling of muscle fibers, cytoplasmic homogenization, and loss of cross-striation (Mikhlin and Fuior 1976).

In a study by Averbukh et al. (1989), two groups of 15 Beit Dagan mice (9 weeks old; average weight = 25 g) received doses of 70 mg/kg and 35 mg/kg p-Phenylenediamine. A significant increase in creatine phosphokinase activity was noted at 24 hours; by 120 hours, the values approached normal levels. Serum aldolase activity increased rapidly and significantly in both groups, but remained significantly increased after 120 hours. In animals killed after 24 hours, acute rhabdomyolysis with segmental necrosis of myofibers (characterized by pyknosis of subsarcolemnal nuclei and fragmentation of the sarcoplasm) was noted. At 120 hours, regeneration and increased numbers of nuclei were observed. Microscopic examination of kidney or liver sections did not reveal any abnormalities.

Yabe et al. (1991) observed rhabdomyolysis in dogs exposed to p-Phenylenediamine . p-Phenylenediamine was administered orally to 14 hybrid dogs at doses of 50, 80, and 100 mg/kg. The dogs presented with marked edema of the face, extremities, and external genitals and painful muscle rigor. Massive necrosis of skeletal muscle was observed at microscopic examination. This finding was most pronounced at the 80 mg/kg dose.

Table 7. Subchronic/Chronic Dermal Toxicity of p-Phenylenediamine.

Material	Concentration	Method	Duration	Animals	Results	Reference
			Subchronic Rabbit Studies			

Material	Concentration	Method	Duration	Animals	Results	Reference
Hair dye composite formulation	1.2%	Repeated treatment group received 1 g/kg daily 5 times a week for 65 applications on shaved abdomen. The skin of 4 rabbits in this group and control group was abraded. The as used group received 10 g/application massaged into their black fur 10 minutes, allowed to remain an additional 20 minutes, then rinsed and dried, once every 2 weeks for 7 exposures	13-14 weeks	5 male and 5 female rabbits in treated and control groups	Accumulation of dye on shaved animals resulted in fissures, scab formation, and desquamation; no observations on unshaved group. Mild to marked acanthosis and mild to moderate dermal fibroplasia in exposed skin of 7 rabbits in repeated test group. No differences between intact and abraded skin. Moderate acanthosis and very mild dermal fibroplasia in exposed skin of 2 animals in as used group. Hematological analyses, clinical blood chemistry, and urinalyses normal. Gross pathology revealed equivocal kidney lesions in test groups; was not confirmed histopathologically	CTFA 1969c
4 hair dye composite formulations	1.0, 2.0, 3.0, or 4.0%	Mixed 1:1 with H_2O_2. 1 mg/kg applied 2 times a week to 2 clipped, alternated sites on the back. Sites on half of the animals were abraded once a week. Rabbits were restrained for 1 hour following dye application and then were shampooed, rinsed and dried	13 weeks	Groups of 12 rabbits, 3 control groups	No evidence of compound-induced toxicity. Body weight gain was normal. Blood and urine parameters and organ weights were not significantly different from controls. No gross abnormalities observed at necropsy. No microscopic lesions due to hair dye administration	Burnett et al. 1976
			Chronic mouse Studies			
3 hair dye experimental formulations	1.50% in the hair dyes	Mixed 1:1 with H_2O_2. 0.05 ml applied weekly or fortnightly to shaved mid-scapular skin	18 months	Groups of 100 mice, 250 control mice	No overt signs of systemic toxicity. Survival time and body weights comparable. Liver weights in the range of normal values. All blood parameters within normal limits. Microscopic examination showed skin and appendages normal. Moderate alopecia in about half of the mice receiving hair dyes weekly	Burnett et al. 1975
2 hair dye formulations	1.50%	Mixed 1:1 with H_2O_2. 0.05 ml applied weekly to clipped intrascapular region and dried with a hair dryer without heat	2 years	28 male and 28 female mice in each group, 76 male and 17 female mice in control group	No skin irritation observed. No significant differences in body weight gains. Survival rate of all mice was erratic	Giles et al. 1976
p-Phenylene-diamine in acetone	5 and 10%	Application of 0.02 ml p-Phenylenediamine 2 times a week to a 1 cm diameter regularly shaved area of interscapular skin	Lifetime	Female mice; 50 per dose and 100 controls	Average lifespan unaffected. Normal behavior and no significant changes in body weight or food intake. No treatment-related ulceration or dermatitis was observed	Stenback et al. 1977
4 hair dye composite formulations	1.0, 2.0, 3.0, or 4.0% in the hair dyes	Mixed 1:1 with H_2O_2. 0.025 ml applied once/week to a clipped intrascapular region. 20 mice from each group killed and necropsied at 7 and at 9 months	21-23 months	Groups of 50 male and 50 female mice, 3 control groups	No differences observed in mean absolute and relative liver and kidney weights, and survival rates	Burnett et al. 1980

Table 7 (continued). Subchronic/Chronic Dermal Toxicity of p-Phenylenediamine.

Material	Concentration	Method	Duration	Animals	Results	Reference
			Chronic Rat Study			
3 hair dye composite formulations	2.0, 3.0, or 4.0% in the hair dyes	Mixed 1:1 with H_2O_2. Applied topically to F_0 generation from time of weaning to the weaning of F_{1A}. F_{1A} received 0.2 ml of the hair dye increased by 0.1 ml weekly to 0.5 ml, 2 times a week on the clipped neck and back. 10 rats from each group killed and necropsied at 12 months	2 years	60 male and 60 female rats per group, 3 control groups (from F_{1A} generation)	Dry skin noted in first few weeks of study in 15-20% of female rats and slightly decreased mean values for total erythrocytes, hemoglobin, and hamatocrit observed in male rats receiving hair dye containing 3% p-Phenylenediamine. No other differences observed in general behavior, appearance, biochemistry, and urinalyses	International Research and Development Corporation 1979
			Chronic Rabbit Study			
p-Phenylene-diamine in acetone	5 and 10%	Application of 0.02 ml p-Phenylenediamine 2 times a week to the inside left ear	85 weeks	Female rabbits; 5 per dose and 5 controls	No observed differences in food intake, weight, behavior, or overall appearance. Survival rate unrelated to treatment. No definite signs of toxicity. No differences in blood and urine parameters. No local skin changes	Stenback et al. 1977

In a study by Munday and Manns (1999), 2 groups of 5 female Sprague-Dawley-derived rats (12 weeks old) received subcutaneous injections of p-Phenylenediamine (in saline) twice daily for 3 days. The two groups received doses of 100 µmol/kg/day and 200 µmol/kg/day, respectively. Necrosis of the gastrocnemius muscle, diaphragm, and tongue was observed in the higher dose group. Muscle lesions were not observed in the lower dose group. At necropsy, macroscopic changes were not observed in either dose group. All of the animals remained in good condition throughout the treatment period.

p-Phenylenediamine HCl

Mascres and Jasmin (1974a,b; 1975) studied skeletal muscle lesions induced in rats (weighing approximately 150 to 200 g) by means of a single, subcutaneous injection of p-Phenylenediamine HCl (3 mg) in aqueous solution (unspecified concentration). Lesions occurred 1 to 24 hours after injection and were characterized by necrosis and edema. In the diaphragm, lesions were accompanied by various myo- and neuromyopathies. Serum activities of creatine phosphokinase and lactic dehydrogenase remained unchanged following the single subcutaneous injection. Two subcutaneous injections of the p-Phenylenediamine solution separated by an interval of 12 hours caused an increase in the serum activity of creatine phosphokinase, but no significant change in the activity of lactic dehydrogenase was observed. Zones of lysed myocardial cells were also observed 24 hours after the 2 doses.

Ocular Irritation

p-Phenylenediamine

A hair dye composite formulation containing 1.2% p-Phenylenediamine was tested for ocular irritation with 10 rabbits. One-tenth ml of the dye was instilled into the conjunctival sac of one eye of each animal. The hair dye was rinsed from the eyes of 5 animals with 40 ml water 4 seconds after instillation. The maximum possible irritation score was 110. For the unwashed eyes, the average irritation score at 1 hour was 33.0, at 24 hours was 34.0, at 48 hours was 24.0, at 72 hours was 14.0, at 96 hours was 9.0, and at 7 days after instillation was 2.4. For the washed eyes at the same times after instillation the scores were 23.0, 20.0, 10.0, 7.0, 4.0, and 0, respectively (CTFA 1969d). A similar study was conducted with a hair dye containing 1.8% p-Phenylenediamine. One-tenth ml was instilled into the conjunctival sac of one eye of 6 rabbits. The maximum possible irritation score was 110. The average scores were 30 for 1 day, 29 for 2 days, 19 for 3 days, 15 for 4 days, and 6 for 7 days after instillation. The hair dye was moderately irritating to the eyes of rabbits (CTFA 1971a).

Morikawa et al. (1976) used the Draize method and scoring system to determine the irritation of 100% p-Phenylenediamine in rabbit eyes. The maximum irritation score reported was 17.0 of a possible 110.

Lloyd et al. (1977) placed 0.1 ml of a 2.5% aqueous p-Phenylenediamine solution containing 0.05% Na_2S0_3 and adjusted to pH 7.0 in one eye of each of 3 rabbits. Ten seconds later the eyes were irrigated with 50 ml of lukewarm water. The researchers observed mild conjunctival inflammation that did not persist for more than 24 hours.

Skin Irritation

p-Phenylenediamine

p-Phenylenediamine and hair dye formulations containing p-Phenylenediamine have been tested for primary skin irritation in a variety of animal species: these include rabbits (Hanzlik 1923; Lloyd et al. 1977; CTFA 1969b; CTFA 1971b; Davies et al. 1972; Herve-Bazin et al. 1977; Morikawa et al. 1976), guinea pigs, mice, miniature pigs, piglets, dogs and baboons (Davies et al. 1972). These studies are summarized in Table 8.

Skin Sensitization

p-Phenylenediamine

Several researchers have proposed that the oxidation products of p-Phenylenediamine were involved in the production of allergic sensitization reactions. It has been suggested that quinonoid intermediates, such as quinonediamine, may link with skin proteins to form antigens (Reiss and Fisher 1974; Blohm and Rajka 1970; Mayer 1955). Hapten-amino acid adducts may be the primary sensitizers in allergic contact sensitization, although the visible allergic reaction would require hapten-protein conjugation (Jansen et al. 1964). These authors indicated that there was no direct experimental evidence that the allergic behavior of p-Phenylenediamines required the binding of quinonoid oxidation products to epidermal proteins.

Evidence presented by Reynolds et aI. (1970) suggested that p-Phenylenediamines differed from many contact allergens; they did not form stable isolatable adducts with epidermal amino acids. In their studies on guinea pig skin epidermis in vivo, binding between highly reactive p-Phenylenediamine oxidation products and skin protein was demonstrated; however, binding was relatively labile and a hapten-amino acid adduct could not be isolated. The authors interpreted this to mean that, if the antigen in p-phenylenediamine hypersensitivity is an epidermal protein conjugate, the conjugate is bound by a much less stable linkage than has been thought necessary.

Experiments have been conducted with p-Phenylenediamine and with hair coloring formulations containing p-Phenylenediamine (Herve-Bazin et al. 1977; Morikawa et al. 1976; Brulos et al. 1977; CTFA 1982b; Goodwin et al. 1981; Kleniewska and Maibach 1980; Magnusson and Kligman 1970; Maurer et al. 1979; Rajka and Blohm 1970; Stevens 1967). p-Phenylenediamine was 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 on test. A hair-coloring formulation containing 2% p-Phenylenediamine did not sensitize any of 12 guinea pigs.

Results of a study with p-Phenylenediamine in the guinea pig lymphocyte transformation test correlated well with sensitivity (Milner 1971). Maguire (1973) reported that a hair dye with 28% p-Phenylenediamine derivatives was a strong guinea pig skin sensitizer.

Sensitization may be transferred from a sensitized guinea pig to a nonsensitized guinea pig by the transfer of intact, sonicated, or disrupted cells (Kind et al. 1965), by the transfer of lymph node or splenic cells (Macher and Atzpodien 1968), or by arteriovenous cross-transfusion or parabiosis (Wahlberg 1979).

Maurer et al. (1984) conducted a sensitization study using 3 different guinea pig strains (Pirbright white, Dunkin Hartley, and Himalayan spotted). Groups of 10 guinea pigs (5 males, 5 females/group) were used in the optimization test. A 3-week induction period (10 intracutaneous applications, 0.1% p-Phenylenediamine) was followed by separate challenges at week 6 (intradermal challenge) and at week 8 (epidermal challenge). The animals were also challenged at 14 days after the last induction. Except for Pirbright white guinea pigs (9 animals challenged), groups of 10 were challenged. Positive reactions were observed in all animals (all strains).

Dossou et al. (1985) studied the sensitization potential of p-Phenylenediamine using groups of 12 female Hartley albino guinea pigs (weights = 300 to 400 g). Two protocols were used to predict and assess allergic reactions. The first protocol involved open epidermal induction and challenge, and the intensity of the reaction was maximized by injecting Freund's complete adjuvant into the foot pad. The second protocol involved a quasi- intradermal induction; both the adjuvant and test substance were injected into the foot pad. In both protocols, the induction concentration of *p*-Phenylenediamine was 0.18 mmol/liter and the challenge concentration was 0.09 mmol/liter. In both protocols, the challenge phase consisted of a single topical application, in the lumbar region, of 10 µl of the test substance. In protocol #1, the sensitization rate was 30%. The sensitization rate was 50% in the protocol #2.

Guillot and Gonnet (1985) conducted an epicutaneous maximization test using 20 adult Albino Dunkin-Hartley guinea pigs. The induction phase involved 7 successive topical applications (48-hour occlusive patches) of 10% aqueous p-Phenylenediamine. On day 28, a 48-hour occlusive challenge patch containing 0.5% aqueous p-Phenylenediamine was applied. Eleven of the 20 guinea pigs were sensitized, and these results were confirmed at microscopic examination.

Table 8. Skin Irritation Studies on p-Phenylenediamine.

Concentration and Vehicle	Method	Animals	Results	Reference
Dry, basic compound	Shaved, washed, and dried a 25 cm^2 area of skin. Rubbed p-Phenylenediamine into skin	3 rabbits	No effects observed	Hanzlik 1923
10% alcoholic solution	Shaved, washed, and dried a 25 cm^2 area of skin. Applied p-Phenylenediamine	3 rabbits	Mild erythema observed	Hanzlik 1923
Hair dye, 1.2%	Approximately 10% of the body surface clipped (the backs). Applied 10 g/kg, wrapped in impervious plastic sheeting for 24 hours	2 male and 2 female rabbits	Slight to moderate erythema and moderate edema observed	CTFA 1969b
Hair dye, 1.8%	Applied 0.1 ml daily for 3 days to intact shaved skin. Graded at 24 hours. PII (scale = 0-8) determined on day greatest irritation observed	9 rabbits	PII = 1.25. Mildly irritating	CTFA 1971b
50%, aqueous slurry	1 24-hour occluded patch application to clipped, intact and abraded skin. Erythema and edema reactions are evaluated (0-4) at 24 and 72 hours, and values are added and averaged to yield a PII (scale = 0-8). Dog and baboon PII are estimates; no abraded sites	6 each of mice, guinea pigs, rabbits, miniature pigs, piglets, dogs and baboons	PIIs for mice, guinea pigs, rabbits, miniature pigs, piglets, dogs, and baboons were 0.8, 0.5, 2.0, 0.4, 1.8, 0.5, and 0, respectively. Rabbit and piglet showed flare-up skin reactions 2 to 3 weeks after application	Davies et al. 1972
5%, ethanol	1 24-hour occluded patch application to clipped skin. The skin of half the animals was abraded. Erythema and edema reactions are evaluated (0-4) at 24 and 72 hours and values are added and averaged to yield a PII (scale = 0-8)	8 rabbits	PII = 0.88	Morikawa et al. 1976
0.5 g/ml, water; 2.5%, petrolatum; 25%, petrolatum; 0.05 g/ml, olive oil	0.5 ml of the water or olive oil or 0.5 ml of the petrolatum applied to intact and abraded skin. Erythema and edema reactions are evaluated (0-4 at 24 and 72 hours, and values are added and averaged to yield a PII (scale = 0-8)	6 rabbits per treatment	PII = 3.2 PII = 1.4 PII = 2.3 PII = 3.4	Herve-Bazin et al. 1977
2.5%, aqueous solution containing 0.5% Na_2SO_3 and adjusted to pH 7.0	1 24-hour occluded patch application to clipped intact and abraded skin. Erythema and edema reactions are evaluated (0-4) at 24 and 72 hours and values are added and averaged to yield a PII (scale = 0-8)	3 rabbits	Very slight edema at the abraded sites of 2 rabbits. In 1, the reaction ameliorated in 72 hours. PII = 0.3	Lloyd et al. 1977

Shigematsu et al. (1988) evaluated the sensitization potential of p-Phenylenediamine using the maximization test. Four inbred strain JY-1 guinea pigs were sensitized with p-Phenylenediamine according to the maximization test methodology, and the animals were challenged with 1% p-Phenylenediamine. All 4 guinea pigs had a positive reaction to 1% p-Phenylenediamine. Cross reactions between 1% p-Phenylenediamine and 1% p-Phenylenediamine derivatives were also observed in this study.

In a guinea pig maximization test performed by Maurer and Hess (1989), both standard and modified protocols were used. In the modified protocol the initial induction consisted of 4 intradermal injections (0.1 ml each) of an adjuvant-saline mixture into the skin of the animals' neck, followed by application of the test substance (in soft white petrolatum) on the injection sites and under occlusion for 24 hours. These were the only modifications to the standard protocol. p-Phenylenediamine was injected in saline. Results for the experiment using the standard protocol (0.5% intradermal induction concentration; 10.0% epidermal induction; 1.0% epidermal challenge) indicated 20/20

positive reactions. Results for the experiment using the modified protocol (10% - induction concentration; 1.0% - challenge) indicated 10/10 positive reactions.

Bronaugh et al. (1994) performed a series of tests using guinea pigs. The first was an open epicutaneous test (OET) using groups of 10 Hartley guinea pigs (males and females). Daily applications of 0.03% p-Phenylenediamine (in ethanol) were made to clipped dorsal skin for 3 weeks. The challenge phase was initiated at day 1 after the induction phase. Three challenge concentrations of the test substance were applied to previously unused sites on the back; reactions were scored 24 and 48 hours later. Sensitization was not achieved, even at the highest possible challenge dose of 10%. When the induction dose was increased to 0.1%, only 6 of 10 animals gave a positive response to p-Phenylenediamine.

These authors also performed a Buehler occlusive patch test using groups of 10 Hartley guinea pigs. Sensitization was induced by 1 weekly topical application of 0.03% p-Phenylenediamine (in ethanol, occlusive patch) to clipped skin for 3 weeks. The challenge and rechallenge occurred at weeks 5 and 6. At the highest challenge concentration (10% p-Phenylenediamine), 9 of 10 guinea pigs had positive reactions.

These authors evaluated the sensitization potential of p-Phenylenediamine in the maximization test using Hartley guinea pigs. Intradermal injections were made on the first day of each week for 3 weeks. For week 1, p-Phenylenediamine was combined (1:1, v/v) with Freund's complete adjuvant. For week 2, p-Phenylenediamine was combined (1:1, v/v) with Freund's incomplete adjuvant. For week 3, only p-Phenylenediamine was used. One week was allowed for the development of sensitization, after which challenge and rechallenge were performed during weeks 5 and 6. The animals were sensitized with 5% p-Phenylenediamine and challenged with doses ranging from 0.01% to 6%.

At the highest challenge concentration (6%), positive responses were observed in 223 of the 250 animals. At the lowest challenge concentration (0.01%), positive responses were observed in 30 of the 200 animals. Microsopic examination of skin reaction sites from p-Phenylenediamine-sensitized guinea pigs revealed marked infiltration of mononuclear cells, classically seen with delayed-type hypersensitivity skin reactions. Acanthosis and mononucleocyte infiltration were reported and eosinophil numbers were significantly increased (Bronaugh et al. 1994). Warbrick et al. (1999) conducted 4 independent analyses of p-Phenylenediamine in the murine local lymph node assay (LLNA), performed in parallel in each of 2 independent laboratories over a period of 4 consecutive months. Proliferative responses were measured at 5 test concentrations (0.05%, 0.1%, 0.25%, 0.5%, and 1%), together with concurrent vehicle control group. Results obtained in each laboratory were expressed as the incorporation of [^{3}H]TdR into lymph nodes draining the site of topical exposure (dpm per node). Chemicals that, at 1 or more test concentrations, caused a threefold or greater increase in proliferation, compared to concurrent vehicle-treated controls, were considered to have the potential for causing skin sensitization. In each experiment and in both laboratories, p-Phenylenediamine provoked a vigorous proliferative response (positive response) at concentrations of 0.25% or greater.

Table 9 summarizes these sensitization studies.

Skin Sensitization/Cross-sensitization

p-Phenylenediamine

Herve-Bazin et al. (1977) reported that 80% of treated guinea pigs were sensitized to 0.05% p-Phenylenediamine in petrolatum. Through crossreactions, 95 to 100% of the treated guinea pigs were also sensitized to 0.5% in petrolatum of these amine antiozonants:

- N-phenyl-N'-cyclohexyparaphenylenediamine (CPPD),
- N-dimethyl-1,3-butyl-N'-phenylpara-phenylenediamine, and
- N-isopropyl-N'-phenylpara-phenylenediamine (IPPD).

Using the guinea pig maximization test, Xie et al. (2000) studied the individual skin sensitization potency and cross-reactivities of:

- p-Phenylenediamine,
- p-toluenediamine · 2 HCl (PTD),
- p-aminophenol (PAP),
- p-aminoazobenzene (PAB), and
- Sudan III (a lysochrome, fat-soluble, diazo dye).

In each group, 6 female Hartley strain albino guinea pigs were induced with 0.1% of the test chemical by intradermal injection and 6 by topical application (1.0% concentration). The animals were challenged with all 5 chemicals (in concentrations of dilution by 10) from 0.1% to 0.001%.

Challenge with the 0.1% concentration yielded the following numbers of positive responses: p-Phenylenediamine (6/6), PTD (6/6), PAP (5/6), and PAB (6/6) groups. Positive responses were not elicited in the Sudan III group. Cross-reactivities to p-Phenylenediamine were confirmed in animals challenged with PTD (6/6), PAP (6/6), PAB (6/6), and Sudan III (3/6).

In the PTD-induced group, positive responses to cross-challenges were elicited by p-Phenylenediamine (5/6), PAP (3/6), PAB (5/6), and Sudan III (1/6). Cross-reactivities to PAP were observed only with p-Phenylenediamine (2/5) and PAB (5/5). PAB-induced animals responded only to p-Phenylenediamine (1/6). Study results indicated that all

chemicals, except for Sudan III, are strong sensitizers. The cross-reactivities to p-Phenylenediamine were higher than those to PTD, PAP, and PAB (Xie et al., 2000).

Table 9. Skin Sensitization Studies on p-Phenylenediamine.

Solvent	Concentration (%)	Method	No. of Animals/cell type	Results	Reference
		Guinea Pigs			
dimethyl formamide	induction with 10; challenge with 1	induction: 0.1 ml applied to outer ears for 3 days; 4 days later, challenge: 0.2 ml applied to depilated flank; erythema scored 24 hours later; controls were challenged, noninduced animals	8 guinea pigs	8/8 positive (pink) reactions	Stevens 1967
propylene glycol for intradermal induction; 70% ethanol for topical induction; and 95% ethanol for challenge	intradermal induction with 0.005, topical induction with 0.05 or 0.1 for larger animals; challenge with 0.5, 1, and 5	guinea pig maximization test; scale 0-3	2 groups of 25 female guinea pigs	14/25 and 16/25 positive reactions. Mean scores were 0.65 and 0.71, respectively	Magnusson and Kligman 1970
water	primary irritation with 0.001, 0.01, 0.1.; induction and challenge with 0.001	primary irritant effect determined after intradermal injection. Induction, intradermal injection over 10 days with 0.1 ml. 17 days later, challenge, intradermal injection of 0.1 ml; reactions read 48 hours later; biopsies performed	20 guinea pigs on test and 18 controls	no primary irritation reactions. 16/20 positive sensitization reactions; high correlation between microscopic and macroscopic reactions	Rajka and Blohm 1970
water	Intradermal induction with 0.1, topical induction with 5, challenge with 1	Guinea pig maximization test; scale 0-3	10 guinea pigs	all animals sensitized; mean erythema + edema score was 2.9	Morikawa et al. 1976
70% ethanol	2	24-h occluded patch of 0.5 ml on back read 1, 7, 24, and 48 hours later; induction with intradermal injection of 0.1 ml Freund's complete adjuvant diluted to 50% in saline on days 0 and 9, occluded patch of 0.5 ml on days 0, 2, 4, 7, 9, 11, 16, 18, and 21 to clipped back; 10^{th} patch removed day 23; challenge on day 35: 48-hour occluded patch of 0.5 ml to another back site; reactions read at 0., 7, 24, and 48 hours; erythema scored on scale of 0 to 4; histological examination, challenge sites with macroscopic reactions	20 guinea pigs	20/20 reacted; mean erythema score was 2.16. 2/3 had allergic type inflammatory reactions with intense spongiosis and massive lymphocyte exocytosis; 1 showed necrosis with erosion, weeping, and a squamous crust	Brulos et al. 1977
olive oil for injection induction, petrolatum for dermal induction and challenge	injection induction with 0.5; dermal induction with 1; challenge with 0.05 and 0.5	Induction with intradermal injection of p-Phenylenediamine and Freund's complete adjuvant. 1 week later, cutaneous application; challenge 1 week later by cutaneous application; control animals challenged only	20 guinea pigs per treatment	0.05 and 0.5% p-Phenylenediamine sensitized 80 and 100% of the animals, respectively. 0.5% p-Phenylenediamine irritating to 20% of the nonsensitized 33 controls	Herve-Bazin et al. 1977

Table 9 (continued). Skin Sensitization Studies on p-Phenylenediamine.

Solvent	Concentration (%)	Method	No. of Animals/cell type	Results	Reference
physiological saline for induction and intradermal challenge; petrolatum for epidermal challenge	intradermal induction and challenge with 0.1; epidermal challenge with 1	induction: 3 intradermal injections of 0.1 ml in a week and 3 intradermal injections of 0.1 ml of 1:1 Freund's adjuvant; saline with p-Phenylenediamine for 2 weeks; after 14 days, challenge: intradermal injection of 0.1 ml into fresh flank site and 24-hour occluded patch 14 days later at a different site; challenge reactions read 24 hours after injection or patch removal	20 guinea pigs	20/20 and 13/20 positive sensitization reactions after intradermal and epidermal challenges, respectively	Maurer et al. 1979
petrolatum	induction with 2; challenge with 0.1, 0.5, 1, and 2	induction, 4 24-hour occlusive patches of 0.5 g on alternative days on clipped flank; after 14 days, challenge on opposite flank in occlusive chamber; reactions scored 24, 48, and 72 hours; erythema scored on scale of 0-3	10 guinea pigs at each of four challenge concentrations	0.5, 1, and 2% sensitized all animals; mean erythema scores were 2.2, 2.1, and 1.5, respectively; 0.1% sensitized 4/10; mean score was 0.5	Kleniewska and Maibach 1980
not given	induction injection with 2.5; challenge application with 5.0	4 simultaneous induction injections at sites overlaying axillary and inguinal lymph nodes; challenge by intradermal injection and open topical application on opposite shaved flanks 14 days later; reactions graded at 24 hours	10 guinea pigs	all guinea pigs were sensitized; a strong sensitizer	Goodwin et al. 1981
not given	intradermal induction with 0.25, topical induction with 0.5, challenge with 0.5	guinea pig maximization test; scale = 0-3	10 guinea pigs	90% of the guinea pigs were sensitized; a strong sensitizer; mean of all positive challenge scores was 2.45	Goodwin et al. 1981
not given	induction injection of 0.25; challenge patch with 1.0	single intradermal injection of p-Phenylenediamine and Freund's complete adjuvant in nuchal region; challenged 12-14 days later with 6-hour occluded chamber application on a shaved flank; reactions graded 18 and 42 hours later (scale = 0-3)	10 guinea pigs	all guinea pigs were sensitized; a strong sensitizer; mean of all positive challenge scores was 2.1	Goodwin et al. 1981
hair coloring formulation	2	material diluted to 1% in propylene glycol. 9 0.1 ml topical inductions on the shaved back over 3 weeks; challenge 2 weeks later at the original and an untreated site; observed at 24 and 48 hours	12 female guinea pigs	No positive reactions were observed; not a contact sensitizer	CTFA 1982b
not given	0.1	3-week induction period (10 intracutaneous applications) followed by separate challenges at week 6 (intradermal challenge) and at week 8 (epidermal challenge); animals were also challenged at 14 days after last induction	Pirbright white guinea pigs (9 challenged) and both Dunkin Hartley guinea pigs and Himalayan spotted guinea pigs (groups of 10) were challenged	positive reactions in all 3 strains	Maurer et al. 1984
water	topical induction with 10 aqueous; challenge with 0.5 aqueous	7 successive topical induction applications (48-h occlusive patches); 48-h occlusive challenge patch applied on day 28	20 adult albino Dunkin-Hartley guinea pigs	positive reactions in 11 of 20	Guillot and Gonnet 1985
not given	induction with 0.18 mmol/liter; challenge with 0.09 mmol/liter	protocol #1: open epidermal induction (in footpad); protocol #2: quasi-intradermal injection (in footpad); both protocols: single topical challenge application (in lumbar region)	groups of 12 female Hartley albino guinea pigs	sensitization rates : 30% (protocol #1) and 30% (protocol #2)	Dossou 1985

Table 9 (continued). Skin Sensitization Studies on p-Phenylenediamine.

Solvent	Concentration (%)	Method	No. of Animals/cell type	Results	Reference
not given	induction concentration not stated; challenge with 1	maximization test	4 inbred strain JY-1 guinea pigs	positive reactions in all animals	Shigematsu et al. 1988
not given	induction with 0.5 or 10; challenge with 1	maximization test with 4 intradermal induction injections of adjuvant-saline mixture, followed by test substance (in soft white petrolatum) application, under occlusion, on injection sites for 24 h; or modified protocol: test substance injected in saline	20 guinea pigs (standard protocol); 10 guinea pigs (modified protocol)	positive reactions in all animals (both protocols)	Maurer and Hess 1989
ethanol	induction with 0.03 and 0.1; challenge concentrations up to 10	open epicutaneous test: daily applications of 0.03% made to clipped dorsal skin for 3 weeks; 3 challenge concentrations (up to 10%) applied to new sites	groups of 10 Hartley guinea pigs	sensitization not achieved with 0.03% induction in all animals, even at 10% challenge concentration; when induction concentration increased to 0.1%, 6 of 10 with positive reactions	Bronaugh et al. 1994
ethanol	induction with 0.03; challenge concentrations up to 10	Buehler occlusive patch test; sensitization induced by one weekly topical application made to clipped skin for 3 weeks; challenge and rechallenge at weeks 5 and 6, respectively	groups of 10 Hartley guinea pigs	at highest challenge concentration (10%), sensitization in 9 of 10 animals	Bronaugh et al. 1994
not given	induction with 5; challenge with 0.01 to 6	maximization test; intradermal induction injections on day 1 of each week; 1 week allowed for sensitization, after which challenge and rechallenge performed during weeks 5 and 6.	Groups of 200 and 250 Hartley guinea pigs	at highest challenge concentrations (6%), positive reactions in 223 of 250 animals; at lowest challenge concentration, positive reactions in 30 of 200 animals	Bronaugh et al. 1994
		LLNA			
not given	proliferative responses measured at 5 test conc. (0.05, 0.1, 0.25, 0.5, and 1)	LLNA performed in parallel over 4 consecutive months. Chemicals that (at 1 or more test concentrations) caused a 3-fold or greater increase in proliferation, compared to concurrent vehicle-treated controls, considered to have the potential for causing skin sensitization.	5 groups of 4 CBA/Ca strain female mice	test substance provoked a vigorous proliferative response (positive response) at concentrations of 0.25% or greater.	Warbrick et al. 1999

Li et al. (1996) evaluated the cross sensitization potential of hair dyes in a modified lymphocyte transformation test using 14 Hartley guinea pigs (9 test and 5 controls). The guinea pigs were sensitized with p-Phenylenediamine using the maximization test procedure. The challenge concentrations were 1% p-Phenylenediamine, 1% *p*-aminophenol, and 5% *m*-Phenylenediamine. Lymph node cells from the animals were cultured with p-Phenylenediamine, p-aminophenol, or m-Phenylenediamine in the presence or absence of epidermal cells. Transformed lymphocyte counts were evaluated by means of ^{3}H-thymidine uptake. Results suggested that there is cross-sensitization relationship between p-Phenylenediamine, *p*-aminophenol, and *m*-Phenylenediamine.

p-Phenylenediamine HCl

Lidén (1988) evaluated the cross-sensitization potential of p-Phenylenediamine HCl using a guinea pig maximization test procedure. Outbred female Dunkin Hartley albino guinea pigs (~20 exposed, ~20 sham-treated controls) were used for each test series. The number of animals per test/control group was never less than 19. Color developing (CD) agents (CD-2, CD-3, and CD-4, all derivatives of p-Phenylenediamine) were used for induction. At challenge with p-Phenylenediamine HCl (0.5% in petrolatum), the test sites were randomly rotated. Reactions were scored blindly

at 48 hours and 72 hours post-application. The minimum criterion for a positive reaction was moderate and confluent erythema.

The incidence of positive challenge reactions to p-Phenylenediamine HCl following induction with either of the color developing agents was as follows: induction with CD-2 (3 of 20 test; 1 of 19 controls), induction with CD-3 (1 of 20 test; 1 of 20 controls), and induction with CD-4 (3 of 21 test; 0 of 20 controls). A few reactions to p-Phenylenediamine HCl were observed; however, the incidence in test animals was not any greater than that observed for sham controls or the petrolatum control. There was no cross-reactivity between either of the 3 color developing agents and p-Phenylenediamine HCl (Lidén, 1988).

Lidén and Boman (1988) evaluated the cross-reactivity of p-Phenylenediamine HCl with color developing agents (p-Phenylenediamine derivatives) using a maximization test. Outbred female albino guinea pigs of the Dunkin-Hartley strain were used (groups of 20). The following chemicals were among the color developing agents that were tested:

- 4-N,N-diethyl-2-methyl-1,4-phenylenediamine · HCl (CD-2) and
- 4-(N-ethyl-N-2-methan-sulphonamidoethyl)-2-methyl-1,4-phenylenediamine · 1.5 H_2SO_4 · H_2O (CD-3).

The test procedure consisted of intradermal injection of the color developer (in Freund's complete adjuvant) on day 0, topical treatment with sodium lauryl sulfate (in petrolatum) on day 6 in the final series, topical induction on day 7, and challenge patch testing with p-Phenylenediamine HCl on day 21. The induction test concentrations for CD-2 were 2.15% and 10.74% (in petrolatum), and 4.37% and 21.83% (in petrolatum) for CD-3. p-Phenylenediamine HCl was tested at a concentration of 1.09% in petrolatum during the challenge phase. Topical induction patches consisted of filter paper mounted on Blenderm®. Finn chambers were used during the challenge phase. Results indicated no cross-reactivity between p-Phenylenediamine HCl and either CD-2 or CD-3. No positive challenge reactions to p-Phenylenediamine HCl were reported (Lidén and Boman, 1988).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In Vivo Studies

p-Phenylenediamine

Burnett et al. (1976) applied hair dye products containing 1.0, 2.0, 3.0, or 4.0% p-Phenylenediamine topically in dose volumes of 2 ml/kg to groups of 20 pregnant rats on Days 1, 4, 7, 10, 13, 16, and 19 of gestation. The rats were shaved 1 day prior to each application, and the dyes were mixed with an equal volume of 6% hydrogen peroxide just before their use. There were 3 negative control groups. The rats were killed on Day 20 of gestation. No biologically significant soft tissue or skeletal changes were observed in the embryos. The mean numbers of corpora lutea, implantation sites, live fetuses, resorptions per pregnancy, or litters with resorptions were not significantly affected by the treatments. The litter sex ratios and pregnant rat weight changes and feed consumption were similar for untreated controls and dye-treated groups.

IRDC (1977) reported a study in which hair dye formulations containing 2, 3, and 4% p-Phenylenediamine were mixed 1:1 with 6% hydrogen peroxide and applied 2 times a week to the clipped backs and necks of groups of 40 male and 40 female rats (the F_0 generation). The initial dose level was 0.2 ml of the dye per application and this was increased by 0.1 ml/application weekly to a dose of 0.5 ml/application. Treatment was continuous through growth, mating, gestation, and lactation to the weaning of the F_{1B}, F_{2B}, and F_{3C} litters of the respective generations. There were 3 control groups.

The dye-treated groups were comparable to control groups in general behavior and appearance, feed consumption, body weight gain, and survival. Treated rats had a few skin reactions throughout the study; these included mild scabbing, fissuring, loss of elasticity, and leathery texture. The treated F_0, F_1, and F_2 parents did not differ from the controls in fertility, gestation survival, and live birth indices. Litter size and body weights of the young were similar.

No treatment-related gross or microscopic lesions were observed in the F_{1B} parental rats or functional observational battery (FOB) weanling rats killed and necropsied during the study. No treatment related gross lesions were observed in the rats that died during the study (IRDC 1977).

Biodynamics (1977) applied a hair dye formulation containing 3% p-Phenylenediamine, mixed 1:1 with 6% hydrogen peroxide, to the clipped backs of 50 female mice 2 times a week for 4 weeks prior to mating and through the mating and gestation periods. There was evidence of mating in 34 treated mice, and 26 became pregnant. Each mouse received 0.05 ml of the dye and hydrogen peroxide mixture at each application. There was evidence of mating in 30 control mice, and 23 became pregnant.

No overt signs of maternal toxicity were observed. One treated animal died prior to gestation; it had a discolored liver and an enlarged spleen. The maternal weight gains and pregnancy and mortality rates of the treated mice were comparable to the controls.

The mean numbers of implantations, live fetuses and resorptions and fetal sex ratios, and numbers of skeletal and soft tissue malformations were similar in treated and control mice. Slightly lower fetal weights were observed in the treated mice, but the mean crown-rump distances were

comparable to the controls. The researchers concluded that there was no evidence of a teratogenic effect. However, there may have been a retarding effect on the ossification process, particularly of the bones of the feet and of the cervical and caudal vertebral centra (Biodynamics 1977).

Biodynamics (1982) applied the same hair dye formulation to the clipped backs of 34 female rabbits 2 times a week for 4 weeks prior to mating and through the mating and gestation periods. Of the 34 rabbits that were mated, 26 became pregnant, and 3 died during the gestation period. The rabbits received 4.0 ml/kg applications of the mixture of hair dye with hydrogen peroxide. Of the 34 control rabbits, 32 were mated, 21 became pregnant, and 6 died during gestation.

As above, no overt signs of maternal toxicity were observed. No adverse effects on pregnancy rates, maternal survival, and body weights were found. Focal alopecia was noted at slightly higher incidences in treated rabbits during the first two thirds of gestation; in the last third of gestation, the incidence of alopecia in control and treated rabbits was similar. The mean numbers of corpora lutea, implantations, live fetuses and resorptions, implantation efficiency, and number of doses with 2 or more resorptions were comparable in control and treated rabbits. There was no evidence of a teratogenic effect. There may have been some evidence of embryotoxicity; the percent of live fetuses was less in the treated rabbits (85.4% in the treated rabbits and 93.8% in the control rabbits), and the fetal sex ratio (male:female ratio of 0.7) was unusually low. However, there was no adverse effect on the weight or length of the fetuses that survived to Day 30 of gestation (Biodynamics 1982).

Huntingdon Research Center (1978) administered an aqueous p-Phenylenediamine solution subcutaneously in a dose of 28 mg/kg to 3 groups of 25 mice on Days 5 to 7, 8 to 10, or 11 to 14 of gestation. There were 25 control mice.

Small increases over the controls in average resorption numbers and percent fetal skeletal variations were observed in the treated groups; the researchers claimed that these increases were within the range of values found for historical controls. The authors concluded that there was no evidence p-Phenylenediamine had an embryotoxic or teratogenic effect (Huntingdon Research Center 1978).

Topham (1980a,b) used the mouse sperm-head abnormality test to determine the effects of chemical interference in spermatozoa differentiation. Groups of 5 hybrid male mice were given 5 daily intraperitoneal injections of p-Phenylenediamine in saline. p-Phenylenediamine was administered to the mice at doses of 5, 10, and 20 mg/kg per day. Doses of 50 and 100 mg/kg per day were toxic. Five weeks later the mice were killed and sperm smears were made. Two hundred fifty sperm-heads were classified as of normal or abnormal morphology. p-Phenylenediamine was not active in this test.

Re et al. (1981) administered p-Phenylenediamine in aqueous solution by gavage in doses of 5, 10, 15, 20, and 30 mg/kg per day on Days 6 through 15 of gestation to groups of 25 pregnant rats. A control group given only water and a pair-fed control group (paired to 30 mg/kg per day group) were also included in the experiment. The rats were killed on Day 20. Feed consumption was significantly reduced during the dosing period for the 20 and 30 mg/kg per day groups.

There were significant decreases in feed consumption for several days during the dosing period for the 15 mg/kg per day group. Rats in the 20 and 30 mg/kg per day groups and the pair-fed controls had significantly reduced weight gains over the dosing period, but these results were not significant when compared to the controls over the 20 days.

Two pregnant rats in the 30 mg/kg per day group died. There were no other maternal deaths.

No significant differences in the numbers of corpora lutea, implantation sites, live fetuses or resorptions, or in fetal weights and male:female ratios were observed. There were no biologically meaningful or statistically significant increases in the numbers of litters or fetuses with soft tissue or skeletal malformations in any of the treatment groups (Re et al. 1981).

Burnett and Goldenthal (1988) conducted a multigeneration reproduction study involving groups of 40 animals of each sex (Sprague-Dawley rats; 6 to 8 weeks old). Three formulations containing p-Phenylenediamine (2%, 3%, and 4%) were tested. The dyes (mixed with 6% hydrogen peroxide) were applied (0.5 ml) topically twice weekly throughout the growth, mating, gestation, and lactation phases of the F_0 parents to the weaning of the F_{1a} and F_{2b} litters.

Application of the hair dyes had no adverse effect on the fertility of males or females, on gestation, lactation and weaning indices. The average number weaned per litter and the mean body weights of the weanlings were comparable among the treated and control groups. The authors concluded that the frequent application of oxidative hair dyes containing p-Phenylenediamine had no adverse effect on reproduction (Burnett and Goldenthal 1988).

In a study by MDS Pharma Services (2005), 3 groups of 25 mated female Sprague-Dawley rats (10 to 13 weeks old; weights: 209 to 292 g) received p-Phenylenediamine (in sterile water, by gavage) on days 6 through 19 of gestation, at doses of 5, 10, and 20 mg/kg/day, respectively. A control group of 25 mated rats received sterile water (vehicle) during the same period.

No unscheduled deaths were reported and there were no

signs of general toxicity during the study. A slightly transient lower mean gestational body weight gain (indicative of very slight maternal toxicity) was observed in 10 mg/kg/day and 20 mg/kg/day dose groups during the first 3 days of treatment. At the terminal necrospsy examination of adult females, no treatment-related macroscopic findings were noted.

In each group, at least 23 females were pregnant. Except for an equivocal increased incidence of early resorptions at the high dose level, there were no differences in pre- or post-implantation data between treated and control groups. Median live litter sizes were comparable between control and treated groups.

Mean fetal weight (accompanied by slightly retarded ossification) and mean gravid uterine weight were slightly lower in the high dose females than in the other groups (results not statistically significant). The fetal sex ratio was comparable between groups. Malformed fetuses were not observed in any of the groups.

The authors noted that the incidences of fetuses with morphological anomalies or variations did not suggest any adverse effects of the test substance. There were no indications of teratogenicity in any group, and it was concluded that p-Phenylenediamine was non-embryo-fetotoxic and that the maternal no-observed-effect level (NOEL) was 5 mg/kg/day. According to the authors, the developmental no-observed-adverse effect level (NOAEL) was 10 mg/kg/day (MDS Pharma Services, 2005).

In Vitro Studies

p-Phenylenediamine

Traul et al.(1981) treated Rauscher leukemia virus-infected rat embryo cells with p-Phenylenediamine (1.85 to 3.2 µg p-Phenylenediamine/5.2 x 10^4 cells) for 72 hours, and cell survival was determined 6 days later. This assay measures the acquisition of attachment independence, which is manifested by increased cell survival rates. p-Phenylenediamine was positive in this test. Viable cell counts were greater after p-Phenylenediamine treatment than after treatment with solvent (unspecified) alone.

p-Phenylenediamine HCl

Johnson and Gable (1983) investigated the *in vitro* hydra (*Hydra attenuata*) assay as a prescreen for developmental toxicity. The dose selection procedure in the current study was such that the low end of the developmental toxicity dose-response curve was closely approximated. Substances affecting development (D), but only at or very near the adult (A) toxic dose level, are not considered as hazards to the conceptus, even though they can induce terata, fetal death, and/or runts. The larger the A/D ratio, the greater the propensity of the test substance for disruption of development, but not for harming the adult. Reportedly, the A/D ratio for the teratogen thalidomide is approximately 60. The authors pointed out that the great majority of substances have ratios that are near unity. An A/D ratio of 1 was reported for p-Phenylenediamine HCl in the hydra assay.

p-Phenylenediamine Sulfate

Jelinek et al. (1985) estimated the embryotoxic potential of p-Phenylenediamine Sulfate (in sterile redistilled water) and a host of other chemicals using the chick embryo screening test. The test procedure was composed of the following two parts: the estimation of the embryotoxicity range and the determination of the embryotoxicity parameters. Fertilized eggs from White Leghorn Fowl or Ross I stock (embryos aged 1.5, 2, 3, and 4 days of incubation) were used. The following embryotoxicity parameters were established: (1) beginning of the embryotoxicity dose range, (2) dose-response and stage-response relationships, (3) proportion of dead and growth-retarded fetuses, and (4) malformation in the survivors.

For the range estimation, each test substance was diluted or suspended in decimal geometric series, starting with a $1:10^2$ dilution (5 mg/0.5 ml solvent) to a dilution of $1:10^6$. After an incubation period that was approximately 40 hours, 3 or 10 µl of each dilution were injected subgerminally into groups of 6 normal embryos with 10 to 14 somite pairs.

For embryotoxicy parameters, 3 or 4 doses approximately at the beginning of the embryotoxicity range (see above) were applied singly to groups of 10 embryos that were incubated for 2, 3, and 4 days under the same conditions. On the second day, the solutions were injected subgerminally. Day 8 marked the end of the incubation period.

Malformations evaluated were as follows: head (exencephaly, microcephaly, partial cranial-vault defects), face (crossed beak, cleft beak, central face hypoplasia), eye (microphthalmia, buphthalmia, colobomas), body wall (coelosomia, umbilical hernia), trunk (hyperlordosis, scoliosis, syndrome of caudal regression), and, after cutting the body wall and the right ventricle of the heart, heart malformations (double-outlet ventricle, interventricular septal defects). Regarding the evaluation of results, the number of dead, malformed, and growth-retarded fetuses (weighing less than 650 mg and bearing no visible malformation, if any) in every group were totaled.

The beginning of the embryotoxicity range for p-Phenylenediamine Sulfate comprised doses of 0.03 to 3.0 µg/embryo. p-Phenylenedianmine induced significant malformations of the body wall with a mean incidence of 0.03 and the heart with a mean incidence of 0.11 (Jelinek et al., 1985).

GENOTOXICITY

p-Phenylenediamine and p-Phenylenediamine HCl

Genotoxicity studies are given in Table 10 and summarized below.

As shown under *Bacterial Assays*, many studies have been conducted on p-Phenylenediamine using the Ames test and modifications of the Ames test. Virtually all p-Phenylenediamine and p-Phenylenediamine and hydrogen peroxide mixtures were nonmutagenic in the Ames test in the absence of metabolic activation. Mostly mutagenic responses, with some nonmutagenic results, were reported with metabolic activation.

As shown under *Mammalian Cell Assays*, these ingredients are mostly positive without metabolic activation, and mostly negative with metabolic activation.

p-Phenylenediamine was not genotoxic in one assay of bacteriophage T4D. In a test using bacteriophage λ DNA, DNA damage in the form of fragments were produced.

In two studies using fruit flies, genotoxicity was reported, but all studies using animals reported no genotoxicity.

Other genotoxicity studies not shown in Table 10 are discussed below.

p-Phenylenediamine Oxidation Products

Shah and Andrews (1979) tested 5 oxidation products of p-Phenylenediamine for mutagenicity in the Ames test, and all were more mutagenic than p-Phenylenediamine.

Table 10. Genotoxicity Studies on p-Phenylenediamine and its HCl Salt.

Test Substance	Strain/Cell Type	Test Protocol	Results	Reference
		Bacterial Assays		
p-Phenylenediamine alone and with H_2O_2	*S.typhimurium* strains TA100, TA97, TA1538, and TA98	spot test (~1 mg) with and without S-9 [a]	p-Phenylenediamine alone had no mutagenic activity with or without S-9; p-Phenylenediamine and H_2O_2 mixture gave a very strong mutagenic response with TA1538 when S-9 was present	Ames et al. 1975
p-Phenylenediamine alone and with H_2O_2	*S. typhimurium* strains TA1535 and TA1538	plate incorporation with and without S-9	p-Phenylenediamine alone was slightly mutagenic with strain TA1538 in the presence of S-9. p-Phenylenediamine and H_2O_2 mix was mutagenic to strain TA1538 in the presence of S-9.	Venitt and Searle 1976
p-Phenylenediamine in buffer; and with H_2O_2	*S. typhimurium* strain TA98	plate incorporation (15-150 µg/plate) with and without S-9	p-Phenylenediamine in buffer and p-Phenylenediamine and H_2O_2 were bacteriostatic without S-9; p-Phenylenediamine in buffer and p-Phenylenediamine and H_2O_2 (50 and 150 µg/plate) were mutagenic in the presence of S-9	Yoshikawa et al. 1976

Table 10 (continued). Genotoxicity Studies on p-Phenylenediamine and its HCl Salt.

Test Substance	Strain/Cell Type	Test Protocol	Results	Reference
p-Phenylenediamine in buffer; and with H_2O_2	*S. typhimurium* strain TA98	plate incorporation (0.003-1346.153 µg/plate) with and without S-9	p-Phenylenediamine in buffer and p-Phenylenediamine and H_2O_2 (0.003 -1346. 153 µg/plate) had no mutagenic activity without S-9; p-Phenylenediamine in buffer and p-Phenylenediamine and H_2O_2 (13.461 and 134.615 µg/plate) were mutagenic in the presence of S-9.	Yoshikawa et al. 1977
p-Phenylenediamine in DMSO	*S. typhimurium* strain TA1538	plate incorporation with S-9 from uninduced rats and mice and S-9 from animals induced with B-naphtholfavone	No mutagenic activity with uninduced S-9. Slight mutagenic activity with induced rat and mouse liver S-9	Dybing and Thorgeirsson 1977
p-Phenylenediamine in DMSO	*S. typhimurium* strain TA1538	plate incorporation with and without liver S-9 from rats induced with phenobarbital	p-Phenylenediamine (50 and 100 µg/plate) was significantly mutagenic in the presence of S-9	Garner and Nutman 1977
3 liquid hair dyes and 2 hair dye powders containing p-Phenylenediamine	*S. typhimurium* strains TA98, TA100, TA1535, and TA1537	plate incorporation with and without S -9	one liquid induced basepair substitutions without S-9; all others induced frameshift mutations with S-9	Bajaj and Notani 1978
25 hair dye preparations containing p-Phenylenediamine	*S. typhimurium* strains TA1535, TA100, TA1537, TA1538, and TA98	25 dyes tested without S-9 and 20 dyes tested with mouse liver S-9 with strain TA98	7 dyes mutagenic to TA1538 and TA98 without S-9 and 13 were mutagenic; 2 suspected of being mutagenic to strain TA98 with S-9	Havova et al. 1978
p-Phenylenediamine in DMSO	*S. typhimurium* strains TA100 and TA98	S-9 from rats induced with polychlorinated biphenyl; preincubation of p-Phenylenediamine and bacteria with and without S-9 at 37°C (for 20 minutes) followed by plate incorporation	p-Phenylenediamine (0.5-2 µmol/plate) was significantly mutagenic to strain TA98 in the presence of S-9	Degawa et al. 1979
p-Phenylenediamine in DMSO	*S. typhimurium* strain TA98	preincubatiion at 37°C for 1 hour with and without S9, followed by plate incorporation. S-9 was from rats, hamsters, and mice induced with polychlorinated biphenyls, 3-methylchol-anthrene, and phenobarbital or uninduced	p-Phenylenediamine was not mutagenic with all hamster S-9 and with S-9 from rats and mice induced with polychlorinated biphenyls and 3-methylcholanthrene	Yoshikawa et al. 1979
p-Phenylenediamine: in water; in 2% NH_4OH, in 2% NH_4OH, and with H_2O_2	*S. typhimurium* strains TA1535, TA100, TA1537, TA1538, and TA98	plate incorporation with and without S-9 from noninduced and induced rats	p-Phenylenediamine (5-1000 µg/plate) was not mutagenic without induced rat liver S-9; p-Phenylenediamine was slightly mutagenic to strains TA1538 and TA98 with induced rat liver S-9. p-Phenylenediamine (250-1000 µg/plate) was not mutagenic to strain TA1538 and TA98 with S-9 from noninduced rat liver; slight increase in revertant colony number with p-Phenylenediamine and NH_4OH and no activity with p-Phenylenediamine, NH_4OH, and H_2O_2 in strain TA98 in the presence of S-9 from induced rat liver	Shahin et al. 1979

Table 10 (continued). Genotoxicity Studies on p-Phenylenediamine and its HCl Salt.

Test Substance	Strain/Cell Type	Test Protocol	Results	Reference
p-Phenylenediamine HCl	*S. typhimurium* strains TA1535, TA100, TA1537, TA1538, and TA98	plate incorporation with S-9 from uninduced and induced rats and mice.	at 0.1-6.6 mg/plate was not mutagenic without activation but was mutagenic in strains TA1535, TA100, TA1538, and TA98 with induced mouse and rat liver S-9; with uninduced rat liver S-9, there was no mutagenic activity with TA1535 and reduced activity with TA1538 and TA98	Dunkel and Simmon 1980
p-Phenylenediamine	*S. typhimurium* strains TA100 and TA98	preincubatiion for 37°C for 1 hour with and without S9, followed by plate incorporation.	p-Phenylenediamine was mutagenic only in strain TA98 in the presence of S-9	Mori et al. 1980
purified p-Phenylenediamine in water; 2 commercial samples of analytical p-Phenylenediamine in water; and with resorcinol in 50% NH_4OH and with H_2O_2	*S. typhimurium* strain TA98	plate incorporation with and without S-9.	Purified p-Phenylenediamine (0-2 mg/plate) produced no significant increase in number of frameshift revertants; both commercial samples of p-Phenylenediamine (0-2 mg/plate) and the p-Phenylenediamine/resorcinol/H_2O_2 mixture (0-1.0 mg/plate) increased the number of revertants in the presence of S-9.	Crebelli et al. 1981
		microtiter fluctuation test with microsomal activation (method of Gatehouse and Delow 1979)	confirmed above results	
p-Phenylenediamine in water and in DMSO	*S. typhimurium* strains TA1538 and TA98	with S-9; fresh and aged (used 0-4 hours after dilution) test material	aqueous solution and the fresh DMSO solution were nonmutagenic with S-9; aged DMSO solutions were mutagenic with S-9	Burnett et al. 1982
p-Phenylenediamine in DMSO	*S. typhimurium* strain TA98	with S-9; test material exposed to Toshiba fluorescent lamps (15W x 2) at 10 cm for 0-4 hours	p-Phenylenediamine solution kept in the dark was not mutagenic with S-9; all solutions illuminated 10 minutes to 4 hours were mutagenic with S-9	Nishi and Nishioka 1982
p-Phenylenediamine HCl (in distilled water)	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538, and *E. coli* strain WP2 urA	plate incorporation procedure at doses up to 6,666 µg/plate with and without metabolic activation	classified as mutagenic in at least one *Salmonella typhimurium* strain with metabolic activation; not possible to determine whether test substance was mutagenic in *E. coli* strain WP2 uvrA	Dunkel et al., 1985
p-Phenylenediamine	*S. typhimurium* strain TA98	test concentrations up to 100 µg/plate, with and without metabolic activation	mutagenic, with metabolic activation only	Lee et al. 1986
p-Phenylenediamine	*S. typhimurium* strains TA98 and TA100	plate incorporation and preincubation protocols, with and without plant and mammalian hepatic S9; test concentrations up to 10,000 µg/plate	not mutagenic, with or without metabolic activation	Gentile et al. 1987
Mixture of p-Phenylenediamine HCl (55 mM), resorcinol (66 mM), and H_2O_2 (3%)	*S. typhimurium* strain TA98	Ames test with metabolic activation	Oxidative mixture not mutagenic. However, same oxidation mixture without resorcinol was mutagenic	Bracher et al., 1990

Table 10 (continued). Genotoxicity Studies on p-Phenylenediamine and its HCl Salt.

Test Substance	Strain/Cell Type	Test Protocol	Results	Reference
p-Phenylenediamine before and after treatment with H_2O_2; oxidized mixtures of m-Phenylenediamine and p-Phenylenediamine HCl in DMSO; and o-Phenylenediamine and p-Phenylenediamine HCl in DMSO)	*Salmonella typhimurium* strain TA98	suspension assay; dose of each chemical/chemical mixture did not exceed 10 µg/plate	mutagenicity was enhanced by H_2O_2 in the presence of metabolic activation; H_2O_2-oxidized m-Phenylenediamine and p-Phenylenediamine and the o-Phenylenediamine and p-Phenylenediamine mixtures classified as potent mutagens with metabolic activation	Watanabe et al. 1990
p-Phenylenediamine	*S. typhimurium* strains TA98 and TA100	plate incorporation test, with and without S-9	at concentrations up to 3,000 µg/plate, mutagenic to TA98 with metabolic activation (frameshift); not mutagenic to strain TA100 (base pair substitution).	Chung et al. 1995
p-Phenylenediamine	*S. typhimurium* strains TA98, TA98NR, TA100, and TA100NR	plate incorporation test at concentrations up to 3,000 µg/plate.	Mutagenic to strains TA98NR and TA100NR with metabolic activation.	Chung et al. 1996
p-Phenylenediamine	*S. typhimurium* strains TA98 and TA100	Ames test (Maron and Ames 1983). Test concentrations of 67 to 1076 µg/plate with and without metabolic activation	Mutagenic to strain TA98 with metabolic activation only; classified as frameshift mutagen. Increased genotoxicity in strain TA98, with metabolic activation, over range of test concentrations	Assman et al. 1997
p-Phenylenediamine HCl (in purified water)	*S. typhimurium* strains: TA98, TA100, TA1535, TA1537, and TA102	plate incorporation at test concentrations up to 5000 µg/plate with S-9	mutagenic to strain TA98 in the presence of metabolic activation	Covance Laboratories, 2005a
p-Phenylenediamine (in purified water)	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102	plate incorporation, with and without S-9, at test concentrations up to 5000 µg/plate	statistically significant ($p < 0.01$) increase in number of revertants in strain TA100 (at 1000 µg/plate, without metabolic activation); no dose-response relationship; statistically significant increase in number of revertants in strain TA98 at 1000 µg/plate ($p < 0.01$) and 5000 µg/plate ($p < 0.005$)	Garrigue et al. 2006
p-Phenylenediamine in purified water	*S. typhimurium* strain TA98	pre-incubation (with metabolic activation) at test concentrations up to 5000 µg/plate	statistically significant, dose-related increase in number of revertants	Garrigue et al. 2006
p-Phenylenediamine in DMSO	*S. typhimurium* strain TA1535/pSK1002	with and without metabolic activation at test concentrations up to 5 mg/ml	positive results with metabolic activation	Yasunaga et al. 2006
		Bacterial Virus Assay		
p-Phenylenediamine	bacteriophage T4D	test concentrations in assay up to 190.4 µg/ml	not mutagenic	Kvelland 1984
		DNA Assays		
p-Phenylenediamine	bacteriophage λ DNA	in vitro double-stranded DNA breaks assay.	p-Phenylenediamine (250 µM) caused DNA fragments between 0.6 ~4 x 10^6 daltons	Yamada et al. 1985
		Fruit Fly Assay		

Table 10 (continued). Genotoxicity Studies on p-Phenylenediamine and its HCl Salt.

Test Substance	Strain/Cell Type	Test Protocol	Results	Reference
p-Phenylenediamine in DMSO and sucrose	*D. Melanogaster*	sex-linked recessive assay; 5.1 and 15.5 mM fed for 3 days to adult males	increase in sex-linked recessive mutations in first assay in which the dye was suspected of being contaminated; repeat test with higher purity was not mutagenic	Blijleven 1977; 1981
p-Phenylenediamine	*D. melanogaster*	zeste white eye mosaic system at test concentrations up to 0.5 mM; white-ivory system at test concentrations up to 5 mM; and wing spot test at test concentrations up to 2 mM.	significant increase in frequency of mutant clones (classified as positive) in each test	Batiste-Alentorn et al. 1995
		Mammalian Cell Assays		
p-Phenylenediamine HCl	nonreplicating male rat hepatocyes	DNA repair test; test material at 0.005 to 0.1 mg/ml primary culture	toxicity seen at 0.1 mg/ml; no DNA repair synthesis at 0.005 to 0.05 mg/ml	Williams et al. 1982
p-Phenylenediamine	Chinese hamster ovary cells	sister chromatid exchanges assay at test concentrations up to 1 x 10^{-3} M with and without metabolic activation	induced sister chromatid exchanges with or without metabolic activation	Lee et al. 1986
p-Phenylenediamine	Chinese hamster ovary cells	chromosome aberrations assay at test concentrations up to 87 µg/ml	dose-related increase in chromosomal aberrations without metabolic activation	Chung et al. 1995
p-Phenylenediamine	Chinese hamster ovary cells	chromosome aberrations assay at test concentrations up to 87 µg/ml	dose-related increase in chromosomal aberrations in absence of metabolic activation	Chung et al. 1996
p-Phenylenediamine HCl (in distilled water)	L5178Y mouse lymphoma cells	forward mutation assay at test concentrations up to 6.5 µg/ml (without metabolic activation) and 250 µg/ml (with metabolic activation)	concentration-related increase in mutagenicity in 2/3 trials without metabolic activation; and 2/3 trials with metabolic activation	Mitchell et al. 1988a
p-Phenylenediamine HCl (in distilled water)	L5178Y mouse lymphoma cells	L5178Y mouse lymphoma forward mutation assay at test concentrations up to 10 µg/ml (without metabolic activation) and 400 µg/ml (with metabolic activation)	test substance induced significant increases in mutant frequency with and without metabolic activation, although the responses were usually larger without metabolic activation and occurred at less than one-tenth the concentrations required with metabolic activation.	Myhr and Caspary1988
p-Phenylenediamine HCl (in distilled water)	L5178Y mouse lymphoma cells	L5178Y mouse lymphoma forward mutation assay at test concentrations up to 20 µg/ml with and without metabolic activation	mutagenic without metabolic activation and nonmutagenic with metabolic activation; lowest concentration at which positive response observed was 5 µg/ml	Caspary et al., 1988
p-Phenylenediamine HCl (in distilled water)	TK6 human lymphoblasts	TK6 human lymphoblast forward mutation assay at test concentrations up to 20 µg/ml	mutagenic without metabolic activation, but not with metabolic activation; lowest concentration at which positive response observed was 20 µg/ml	
mixture of p-Phenylenediamine HCl (55 mM), resorcinol (66 mM), and hydrogen peroxide (3%)	mouse lymphoma cells	mouse lymphoma assay	oxidative mixture not mutagenic.	Bracher et al., 1990

Table 10 (continued). Genotoxicity Studies on p-Phenylenediamine and its HCl Salt.

Test Substance	Strain/Cell Type	Test Protocol	Results	Reference
mixture of p-Phenylenediamine HCl (55 mM), resorcinol (66 mM), and hydrogen peroxide (3%); mixture of p-Phenylenediamine HCl (55 mM) and hydrogen peroxide (3%)	human lymphocytes and Chinese hamster ovary cells	chromosome aberration assay with and without metabolic activation	human lymphocytes: increase in chromosome aberrations with resorcinol, but not without; Chinese hamster ovary cells: chromosome aberrations and sister chromatid exchanges with and without resorcinol, but no clear reproducible manner, and only at cytotoxic levels	
p-Phenylenediamine HCl (in water)	Chinese hamster ovary cells	hypoxanthine-guanine phosphoribosyl transferace mutation assay at test concentrations of 5 to 30 µl (without metabolic activation) and 100 to 700 µg/ml (with metabolic activation)	test substance induced micronuclei without, but not with, metabolic activation	Oshiro et al., 1991
p-Phenylenediamine HCl (in purified water)	L5178Y mouse lymphoma cells	mutation (at hprt locus) assay using the microtitre fluctuation technique at concentrations up to 80 µg/ml (without metabolic activation) and up to 900 µg/ml (with metabolic activation); and at concentrations up to 60 µg/ml (without metabolic activation) and up to 1000 µg/ml (with metabolic activation)	test substance was not mutagenic in the presence or absence of metabolic activation	Covance Laboratories Ltd., 2005b
p-Phenylenediamine (in purified water)	heterozygous (tk^+/tk^-) L5178Y mouse lymphoma cells	mouse lymphoma assay at test concentrations up to 35 µg/ml (without metabolic activation) and up to 900 µg/ml (with metabolic activation)	not mutagenic with or without metabolic activation; maximum doses were highly toxic	Garrigue et al. 2006
p-Phenylenediamine (in purified water)	Human peripheral blood lymphocytes	micronucleus assay at concentrations up to 1600 µg/ml and 5000 µg/ml with and without metabolic activation, respectively; concentrations up to 2000 µg/ml and 125 µg/ml with and without metabolic activation, respectively	micronuclei induced with metabolic activation, but not without, when tested following 24-hour phytohemagglutinin (PHA) mitogen stimulation; when tested after 48-hour PHA mitogen stimulation, test substance induced micronuclei both with and without metabolic activation	Garrigue et al. 2006
p-Phenylenediamine	Human urothelial cells (SV-HUC-1 cells)	Comet assay. Association of genotoxicity with expression of p53 and cyclooxygenase-2 (COX-2) oncoproteins also studied	dose-dependent DNA damage over dose range: 2 to 450 µg/ml; also induced overexpression of mutant p53 and COX-2 oncoproteins in dose-dependent manner over the same dose range	Huang et al. 2007
		Animal Assays		
p-Phenylenediamine at 0.2% in water	male rats	dominant lethal study; material given i.p. 3x per week for 8 weeks at 2, 6, an 20 mg/kg; mated to unexposed females	no evidence of post-implantation fetal loss	Burnett et al. 1977
p-Phenylenediamine in 0.5% tragacanth gum with 0.05% sodium sulfite	rats	bone marrow micronucleus test; material fed twice at 500 mg/kg	no clear evidence of mutagenicity	Hossack and Richardson 1977
p-Phenylenediamine	male mice	testicular DNA synthesis; material fed once at 200 mg/kg	testicular DNA synthesis depressed	Seiler 1977
dye product with 2.2% p-Phenylenediamine, mixed with H_2O_2	male rats	heritable translocation study; material painted on 2x per week for 10 weeks; mated to unexposed females	numbers of implantations, dead fetuses, and resorptions not different between test and control	Burnett et al. 1981

Table 10 (continued). Genotoxicity Studies on p-Phenylenediamine and its HCl Salt.

Test Substance	Strain/Cell Type	Test Protocol	Results	Reference
p-Phenylenediamine HCl (in distilled water)	CD-1 mice	micronucleus test. 3 groups of CD-1 mice received single i.p. doses of 25, 50, and 100 mg/kg. The animals were killed and bone marrow smears prepared. Polychromatic erythrocytes scored for incidence of micronuclei	no significant dose- or sampling time-related response in micronuclei induction	Soler-Niedziela et al., 1991
p-Phenylenediamine HCl	male F344 rats	rat hepatocyte replicative DNA synthesis (RDS) assay; test substance administered orally or by s.c. injection to 2 groups at doses of 38 and 75 mg/kg	no replicative DNA synthesis induced	Uno et al., 1994
p-Phenylenediamine HCl	male B6C3F$_1$ mice	RDS assay; test substance administered by single oral gavage to 2 groups at doses of 75 mg/kg and 38 mg/kg	no clear positive evidence of replicative DNA synthesis induction	Miyagawa et al. 1995
p-Phenylenediamine HCl	F344 rats and B6C3F$_1$ mice	RDS assay. Doses of 75 mg/kg and 38 mg/kg.	no replicative DNA synthesis induced	Yoshikawa, 1996
p-Phenylenediamine HCl (in olive oil)	male ddY mice; cells in the following organs studied: stomach, colon, liver, kidney, bladder, lung, brain, and bone marrow	Comet assay at a single oral dose of 75 mg	test substance did not yield a statistically significant increase in DNA damage in any of the organs that were studied.	Sasaki et al., 1999
p-Phenylenediamine in deionized water	groups (5 males + 5 females) received single doses of 25, 50, and 100 mg/kg body weight	micronucleus assay (rat bone marrow cells)	no cytogenetic damage leading to micronucleus formation	RCC Cytotest Cell Research GmbH 2006a
p-Phenylenediamine in deionized water	Wistar Hanlbm: WIST (SPF) rats received single oral doses of 50 and 100 mg/kg body weight	in vivo unscheduled DNA synthesis assay (rat hepatocytes)	no DNA damage leading to unscheduled DNA synthesis	RCC Cytotest Cell Research GmbH 2006b

[a] S-9 was prepared from livers of rats exposed to Aroclor unless otherwise specified.

Bandrowski's base and p-nitroaniline were positive in *Salmonella typhimurium* strain TA1538 with metabolic activation; 4,4-azodianiline and p-dinitrobenzene were positive in *Salmonella typhimurium* strains TA1538 and TA100 with metabolic activation; and 2-(4'aminoaniline)-5-hydroxy-1,4-quinonediimine was positive in strain *S. typhimurium* TA1538 without metabolic activation. A solution of p-Phenylenediamine oxidized with hydrogen peroxide in the presence of m-amines, such as 2,4-toluenediamine and 2,4-diaminoanisole, was more mutagenic than a solution of p-Phenylenediamine alone (Shah and Andrews (1979).

Genotoxicity Assays Using Urine From Exposed Animals

p-Phenylenediamine

An aqueous solution of p-Phenylenediamine was administered intraperitoneally to groups of 20 male rats in doses of 2, 6, and 20 mg/kg 3 times a week for 8 weeks. Their urine was tested in 10% DMSO in the Ames test with strain TA1538. Compared to the DMSO vehicle their urine was not mutagenic (Burnett et al. 1977).

Crebelli et al. (1981) performed the Ames test with strain TA98 with and without metabolic activation on urine concentrates from rats treated topically with p-Phenylenediamine/resorcinol conjugates. The urine concentrates induced mutations only with metabolic activation. The urine concentrates of untreated rats did not induce mutations. Fifteen women collected their urine before and after using hair dyes containing 0.46 to 2.55% p-

Table 10 (continued). Genotoxicity Studies on p-Phenylenediamine and its HCl Salt.

Phenylenediamine. The urine was tested in DMSO in the Ames test in strain TA1538 with metabolic activation. The urine was not more mutagenic after hair dye application than before hair dye application (CTFA 1982a; Burnett et al. 1979).

Effect on Gene Expression

p-Phenylenediamine

Hirota and Moro (2006) assessed changes in the gene expression profile of a monocytic leukemia cell line (THP-1) following exposure to p-Phenylenediamine and other chemicals, using oligo-DNA microarrays. p-Phenylenediamine (45 μg/ml) had no effect on gene expression, whereas, 2,4-dinitrochlorobenzene (DNCB, 3 μg/ml) and nickel sulfate (300 μg/ml) did. The doses tested were the doses that were selected for stimulation of THP-1cells. Upregulation of MIP-1β mRNA expression was detected in both DNCB-treated and nickel sulfate-treated THP-1 cells. The secretion of MIP 1β from THP-1 occurred after 24 hours of treatment with DNCB or nickel sulfate, while p-Phenylenediamine had no effect.

DNA Binding

p-Phenylenediamine HCl

In a study by Ioannou and Matthews (1985), a single dose of p-Phenylenediamine HCl (600 μmol/kg [500 μCi/ml/kg]) was administered to male and female Fischer 344 rats (8 to 10 weeks old; weights = 180 to 200 g) and male and female $B6C3F_1$ mice (6 to 8 weeks old; weights = 18 to 25 g).

The animals were killed at 16 hours 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 HCl-derived radioactivity. There was no evidence of covalent binding of p-Phenylenediamine HCl or metabolites with hepatic DNA at the level of detection (1 pmol/mg DNA). Protein-bound p-Phenylenediamine HCl 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 in the course of normal protein turnover.

CARCINOGENICITY

Clayson and Garner (1976) observed that N-hydroxylation was a prerequisite for carcinogenicity of aromatic amines. Evidence reviewed by these authors suggested that aromatic amine carcinogenesis is the result of bioactivation to the ultimate carcinogenic form and then dissociation of the reactive species to give a positively charged ion as represented in Figure 7.

Thuraisingham and Nilar (1980) stated that the resulting electrophilic metabolite may subsequently react covalently with nucleophilic sites on critical macromolecules, such as DNA.

Cell Transformation Studies

Table 11 presents the 3 cell transformation studies. In summary, 2 of the 3 studies failed to find any cell transformation, but one study did report type III foci.

In Vivo Carcinogenicity Studies

p-Phenylenediamine

Saruta et al. (1958) reported that the oral administration of p-Phenylenediamine did not result in any malignant tumors in rats after three exposures. p-Phenylenediamine was administered daily for 8 months, at 0.06 and 0.3 mg, to groups of 5 rats of each sex. p-Phenylenediamine, at 10 mg, was administered daily for 8 months to 5 rats, mg, and these rats were compared to 5 controls. p-Phenylenediamine, at 30 mg, was administered daily to 4 rats; 3 of these rats died before the experiment ended.

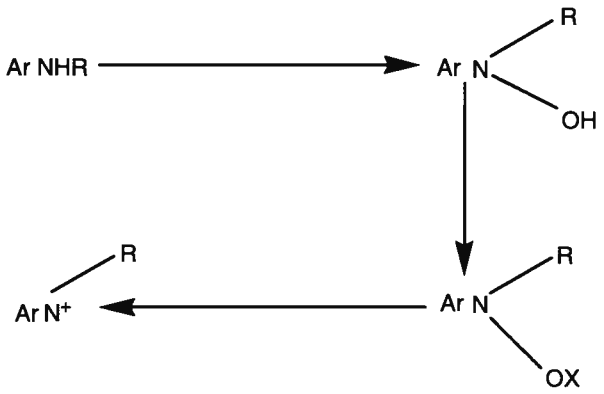

Figure 7. Scheme for metabolic activation of aromatic amines where Ar denotes an aryl group, R is a hydrogen or an acyl or alkyl group, and x is an ester group (Clayson and Garner 1976).

Burnett et al. (1975) conducted a study in which 3 hair dye formulations containing 1.5% p-Phenylenediamine were mixed with equal volumes of 6% hydrogen peroxide just prior to use, and 0.05 ml of each was applied topically to the shaved midscapular skin of groups of 100 mice weekly or every two weeks for 18 months. There were 250 control mice. No evidence of carcinogenic activity by the hair dyes was observed.

Giles et al. (1976) mixed 2 hair dye formulations containing 1.5% p-Phenylenediamine with an equal volume of 6% hydrogen peroxide just before use, and 0.05 ml was applied topically to the clipped intrascapular skin of groups of 28 male and 28 female mice weekly for 2 years. There were 76 male and 17 female control mice. Male and female mice in all groups developed both benign and malignant neoplasms, but there was no difference between control and test animals.

Stenback et al. (1977) conducted a lifetime cutaneous application study using mice. p-Phenylenediamine at 5 and 10% in acetone was applied (0,2 ml) 2x per week to the shaved intrascapular skin of groups of 50 mice. There were 100 untreated controls. Tumors were observed both in the controls and in the treated mice, but there was no significant difference between treated and control animals.

The same investigators applied the p-Phenylenediamine solutions to the inside of the ears of groups of 5 female rabbits 2 times a week. Five control rabbits were included in the study. The experiment was terminated at 85 weeks, but at 80 weeks there were only 2 surviving rabbits in the control group, 4 surviving rabbits in the 5% p-Phenylenediamine-treated group, and 1 surviving rabbit in the 10% p-Phenylenediamine-treated group. No neoplasms were observed (Stenback et al. 1977).

IRDC (1979) conducted a study in which 3 hair dye composite formulations containing 2, 3, and 4% p-Phenylenediamine were applied topically to rats (the F_0 generation) from the time of their weaning to the weaning of their young (the F_{1A} generation). The hair dyes were mixed 1:1 with hydrogen peroxide and were applied topically two times a week for 2 years to the clipped backs and necks of groups of 60 male and 60 female rats of the F_{1A} generation. The rats received an initial application of 0.2 ml, and this was increased by 0.1 ml weekly to 0.5 ml. Ten rats from each group were killed and necropsied at 12 months, and all other rats were necropsied at the time of their deaths or termination of the experiment. There were 3 control groups.

No compound-related gross lesions were observed. The stratum corneum of the skin and of the hair shafts of the treated rats was colored by the dye.

Table 11. Cell Transformation Assays.

Test Substance	Strain/Cell Type	Test Protocol	Results	Reference
p-Phenylenediamine HCl in acetone or DMSO	C3H/10T1/2 clone 8 mouse embryo cell line	C3H/10T1/2 cell transformation assay; exposures from 0.8 to 100 µg/ml in one laboratory and from 0.5 to 5 µg/ml in a second laboratory	inactive in both laboratories	Dunkel et al. 1988
p-Phenylenediamine HCl	BALB/c-3T3 cells	cell transformation assay, exposures up to 0.11 mM for 48 h	cytotoxicity was observed and the test material was considered positive for type III foci	Matthews et al. 1993b
p-Phenylenediamine HCl (pH 6.7)	Syrian hamster embryo (SHE) cells	SHE cell transformation assay; exposures up to 28 µg/ml	50% cytotoxicity at 28 µg/ml and 51% at 12 µg/ml; no significant transformation	Kerckaert et al. 1998

The female rats treated with the composite that contained 4% p-Phenylenediamine had an increase in pituitary adenomas when compared statistically with all 3 control groups (adenomas/number females examined were 34/50, 36/51, and 35/50 for the 3 control groups and 45/51 for the treated group). The authors noted that pituitary adenomas have a high background incidence in rats, and they appeared in a nonsignificant pattern in all the other groups. Other lesions were seen in all the groups in low incidences (IRDC 1979).

Burnett et al. (1980) mixed (1:1) hair dye composite formulations containing 1, 2, 3, and 4% p-Phenylenediamine with hydrogen peroxide, and 0.025 ml of the dyes was applied topically to the clipped intrascapular areas of groups of 50 male and 50 female mice once weekly for 21 to 23 months. At 7 and 9 months, 10 male and 10 female mice from each group were killed and necropsied. Gross and microscopic examinations were made on all mice that died during or were killed at the termination of the experiment. There were three control groups. The incidences of tumors in control and treated groups were similar. The authors concluded that carcinogenic effects were not induced by the hair dye formulations.

In a carcinogenicity study by Imaida et al. (1983), p-Phenylenediamine (0.1% and 0.05% in the diet) was fed to 2 groups of 63 to 66 F344 rats of each sex (6 weeks old), respectively, for 80 weeks.

The body weight of female rats given 0.1% p-Phenylenediamine was less than that of the controls, but no differences were noted at 0.05% or at either exposure in males.

In both sexes, the highest incidence of neoplastic lesions was that of pheochromocytomas of the adrenal gland. These lesions were observed in 10 (27.8%) of 36 male rats given 0.1% p-Phenylenediamine, 8 (22.9%) of 35 male rats given 0.05% p-Phenylenediamine, and 6 (31.6%) of 19 males in the control group, but there was no significant difference in their incidences in different groups. In females, pheochromocytomas were also found in all experimental groups, although at lower incidences than in males. Other neoplastic lesions were as follows: 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. The incidences of these lesions were not significantly different in different groups.

The incidences of non-neoplastic lesions, including hemorrhage of the pituitary gland, fatty degeneration of the liver, fibrosis of the pancreas, and pneumonia were also not significantly different in different groups. No marked changes of the thyroid gland were observed in any rats. The authors concluded that, in this study, p-Phenylenediamine was not carcinogenic to F344 rats of either sex when given orally for 80 weeks (Imaida et al. 1983).

In a study by Burnett and Goldenthal (1988), 3 formulations containing p-Phenylenediamine (2%, 3%, and 4%, respectively) were tested in a multigeneration reproduction study, with a carcinogenicity arm. The dyes were mixed with 6% hydrogen peroxide and applied (0.5 ml) topically twice weekly during the growth, mating, gestation, and lactation phases of the F_0 parents to the weaning of the F_{1a} and F_{2b} litters. In the carcinogenicity arm, 60 male and 60 female weanling rats (randomly selected from each group of F_{1a} litters of the reproduction study) received topical applications of the same test formulations as their parents for approximately 2 years.

No treatment-related gross lesions were observed in any animals necropsied at month 12 or at study termination, or in rats that died during the study. Comparison of tumor indices among the six treated and three control groups showed some significant variations among those tumors occurring most frequently in this strain of rats, and pituitary adenomas were also increased significantly ($P < 0.05$) in the females of one of the treated groups.

That no pituitary carcinomas occurred in this group suggested to the authors that the distribution of these tumors was not related to the experimental treatments. None of the increases in pituitary tumors reported in this study were found to be consistently significant, using $P < 0.01$, the value deemed necessary by statisticians at the National Toxicology Program (NTP) to avoid false positive results. The authors concluded that the frequent topical application of oxidative hair dyes containing p-Phenylenediamine and other commonly used intermediates and couplers does not increase the risk of developing cancer (Burnett and Goldenthal, 1988).

PreClinical Safety Consultants Limited (2005) provided an expert opinion on the carcinogenic potential of p-Phenylenediamine, noting 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 showed that PPD cannot be converted to reactive N-hydroxyarylamine metabolites that are implicated in bladder carcinogenesis.

p-Phenylenediamine HCl

The National Cancer Institure (NCl) administered p-Phenylenediamine HCl in the feed at concentrations of 625 and 1250 ppm to groups of 50 rats and mice of each sex for 103 weeks (NCI 1979). The controls were groups of 20 animals of each species and sex. At the conclusion of the experiment, all animals were killed and necropsied. Both treated and control rats had a variety of neoplasms distributed almost equally between treated and control rats. For each sex the tumor incidence was very low and was within the range normally encountered in aging rats. A variety of tumors, all previously reported to occur spontaneously in mice, were found in both the control and dosed mice. Some neoplasms did occur only, or in greater frequency, in the dosed groups, but none was considered compound-related. The researchers concluded that p-Phenylenediamine HCl was not carcinogenic to rats or to mice under the conditions of the bioassay.

In an evaluation of the carcinogenic risks of p-Phenylenediamine to humans (IARC 1987), the IARC ad-hoc Working Group concluded that p-Phenylenediamine is not classifiable as to its carcinogenicity to humans. It is important to note that the same conclusion was published by IARC in 1978; actually, carcinogenicity data on p-Phenylenediamine HCl were evaluated (IARC 1978).

Regarding IARC's initial conclusion, the Working Group noted that p-Phenylenediamine has been inadequately tested in mice by skin application and in rats by oral and subcutaneous administration, and that studies in mice in which p-Phenylenediamine as a constituent of hair-dye preparations was tested by skin application cannot be evaluated. The Working Group also noted that no case reports or epidemiological studies were available to the Working Group.

The results from the NCI bioassay on p-Phenylenediamine HCl are discussed in a publication by Sontag (1981). The author 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.

In another publication, Prival and Dunkel (1989) stated that in view of the study results and failure to achieve a maximum tolerated dose in male mice, it seems unreasonable to conclude from the NCI carcinogenesis bioassay study that p-Phenylenediamine HCl has been definitely shown to be noncarcinogenic.

Maronpot et al. (1986) evaluated the potential of p-Phenylenediamine HCl to induce lung tumors in strain A/St mice at 2 different laboratories. p-Phenylenediamine was subjected to blind testing. At the first laboratory, groups of A/St mice (10 males/10 females per group; 6 to 8 weeks old) were injected i.p. with high, ½ high, or 1/4 high doses of each test substance, respectively, three times per week for 8 weeks. The dose volume per injection was 0.1 ml/mouse. The high dose selected was the maximum dose of a chemical that did not cause death, growth retardation, or overt toxicity during the preliminary dose-setting phase of the study. p-Phenylenediamine HCl (in tricaprylin) was tested at doses of 6.25, 12.5 and 25 mg/kg per injection. The control groups consisted of untreated mice, vehicle control mice (dosed with tricaprylin), and positive control mice (dosed with urethan).

At the second laboratory, groups of A/J mice (30 males per dose per chemical) were injected i.p. with high, ½ high, and 1/5 high doses 3 times per week for 8 weeks. The dose volume per injection was 0.1 ml/10g body weight. p-Phenylenediamine HCl (in corn oil vehicle) was tested at doses of 6.4, 16, and 32 mg/kg per injection. The control groups consisted of untreated mice, vehicle control mice (dosed with corn oil or saline), and positive control mice (dosed with urethan).

At both laboratories, dosing was followed by a 16-week

incubation period and the animals were killed at the end of the study. Lungs were removed and tumors counted 48 hours after placement in a fixative Microscopic examination confirmed several representative tumors to be alveolar-bronchiolar adenomas. A statistically significant ($p < 0.05$) increase in both the incidence of tumor-bearing mice and tumor multiplicity was necessary for the classification of results as positive. If only 1 of the 2 was statistically significant, the outcome was designated as equivocal.

Overall, test results from the first laboratory for p-Phenylenediamine HCl in tricaprylin were negative in male A/St mice and equivocal in female A/St mice. Though the results for p-Phenylenediamine HCl were equivocal in female mice, the number of tumors per mouse was significantly different ($p < 0.05$) only at a dose of 25 mg/kg when compared to the vehicle control group. For all dose levels, values for the percentage of survivors with tumors and the number of survivors were not significantly different from the vehicle control group.

The test results in the second laboratory (male A/J mice tested) indicated that for p-Phenylenediamine HCl in saline (all dose levels), values for tumors per mouse, percentage of survivors with tumors, and number of survivors were not significantly different from vehicle controls. The results for p-Phenylenediamine HCl were classified as negative (Maronpot et al., 1986).

Rojanapo et al. (1986) evaluated the carcinogenicity of p-Phenylenediamine HCl (commercial sample) using Wistar rats (4 groups of 10 males and 10 females; weights = 120 to 140 g). The test substance was applied topically to shaved skin and was also injected subcutaneously (s.c.). In group 1, the test substance (0.5 ml of a 1:1 mixture of 5% p-Phenylenediamine, in 2% NH_4OH) and 6% H_2O_2 were painted on to shaved skin of the back once per week for 18 months. Group 2 animals were injected s.c. with 0.1 ml of a 1:1 mixture of 5% p-Phenylenediamine (in 2% NH_4OH and 1.8% NaCl) and 6% H_2O_2 every other week for 18 months. Groups 3 and 4 (controls) were dosed with vehicle only (topical application and s.c. injection vehicles, respectively).

In female rats, both topical application and s.c. injection of oxidized p-Phenylenediamine HCl for 18 months induced a statistically significant incidence of mammary gland tumors (> 50%, $P < 0.05$). Additionally, uterine tumors and soft tissue tumors of both malignant and benign types were also significantly induced (43% and 57%, $P < 0.05$) in the s.c. injection group. Tumors of mammary gland and soft tissue were not observed in male rats under similar experimental conditions. However, tumors of other organs, including liver, kidney, adrenal gland, thyroid gland, urinary bladder, and lung were occasionally observed in male rats of both groups and might be related to p-Phenylenediamine HCl treatment (Rojanopo et al., 1997).

Sakai et al. (2002) evaluated the carcinogenic potential of p-Phenylenediamine HCl using a medium-term bioassay system that was based on the induction of glutathione S-transferase placental form (GST-P) positive liver cell foci in rats. According to Low-Baselli et al. (2000), GST-P positive cells can be considered initiated and capable of evolving into hepatic preneoplastic lesions. Additionally, according to Tatematsu et al. (1988), GST-P positive cells develop rapidly into GST-P positive foci, and the foci can be stimulated strongly by feeding 2-acetylaminofluorene (2-AAF) to partially hepatectomized animals.

A single dose of p-Phenylenediamine HCl (40 mg/kg, in saline) was administered to 14 male Fischer 344 rats (7 weeks old; weights = 150 to 170 g) intragastrically (i.g.) at 12 hours after partial hepatectomy. The 18 control animals received saline i.g. The animals were fed a basal diet for 2 weeks, after which they were placed on a diet containing 0.015% 2-AAF for the next 2 weeks. At three weeks after partial hepatectomy, all of the animals received carbon tetrachloride (CCl_4, 0.8 ml/kg body weight, i.g.) as a stimulus for proliferation. After week 5, the survivors were killed and livers were excised and prepared for the immunohistochemical examination of GST-P positive foci. Compared to the negative control, p-Phenylenediamine HCl did not cause a significant increase in the number of GST-P positive foci. However, the following five genotoxic hepatocarcinogens caused significant induction of GST-P positive foci: 2-AAF, diethylnitrosamine, dimethylnitrosamine, N-bis(2-hydroxypropyl)-nitrosamine, and safrole (Sakai et al., 2002).

Modification of Carcinogenicity

p-Phenylenediamine

Hagiwara et al. (1990) investigated the modifying effects of p-Phenylenediamine on liver carcinogenesis in male F344/DuCrj rats initially treated with N-nitrosodiethylamine (DEN). A total of 150 male rats (6 weeks old) was used. Two weeks after administration of a single dose of DEN (200 mg/kg, i.p.), groups of 25 rats were given 1000, 330, and 110 ppm p-Phenylenediamine in diet, respectively, for 6 weeks. 3'-Methyl-4-dimethylaminobenzene (positive control: 3'-Me-DAB, 600 ppm) was also administered in the diet for 6 weeks. At week 3 following DEN administration, all of the animals were subjected to partial hepatectomy.

Slight retardation of body weight was observed in rats treated with p-Phenylenediamine (all dietary levels). Significant increases in relative liver weight were reported for animals treated with 1000 ppm p-Phenylenediamine. Growth retardation and increased liver weight were reported for rats fed 3'-Me-DAB in the diet.

p-Phenylenediamine did not significantly increase the level of γ-glutamyl transpeptidase (γ-GT) positive foci that were observed after DEN initiation. In contrast, 3'-Me-DAB

induced marked enhancing activity, as evidenced by significantly increased values for γ-GT positive foci, compared to controls given DEN alone. The authors concluded that these results demonstrated that p-Phenylenediamine does not modify liver carcinogenesis in this assay system (Hagiwara et al. (1990).

CLINICAL ASSESSMENT OF SAFETY

Dermal Metabolism

p-Phenylenediamine

Kawakubo et al. (2000) investigated the capacity of human skin for N-acetylation of p-Phenylenediamine. In human skin samples (obtained through mammoplasty reduction), p-Phenylenediamine was acetylated to monoacetyl-p-Phenylenediamine (MAPPD), which in turn was acetylated to N,N'-diacetyl-p-Phenylenediamine (DAPPD). This was determined using cytosolic fractions from human skin (n = 9) and cultured normal human epidermal keratinocytes (n = 7).

Nohynek et al. (2004) profiled urinary [14C]-metabolites and N-acetyltransferase 2 (NAT2) genotype in 8 male subjects (mean age: 25 ± 9 years; mean body weight: 78.5 ± 10.0 kg), after treatment with a dark-shade oxidative hair dye containing [^{14}C]-p-Phenylenediamine. The oxidative dye (70 ml; corresponding to a mean of 1.31 ± 0.05 geq. [^{14}C] - PPD per subject) was applied to the hair for 30 minutes. Application was followed by rinsing and washing with water and shampoo. The [^{14}C]-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 hours following hair dye treatment at 4-hour intervals (up to 14 hours), followed by collection at 12-hour 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 [^{14}C] in the urine within 24 hours after treatment, and MAPPD and DAPPD (considered major urinary metabolites, present in all urine samples; accounted for 80 to 95% of the total urinary [^{14}C]) 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 [^{14}C]). 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 (Nohynek et al. 2004).

Contact Urticaria

p-Phenylenediamine

Fuchs and Wahl (1992) tested 31 patients (20 to 53 years of age) with contact urticaria and/or systemic reactions to different latex products to different allergens. Most of the patients were considered to be atopic based on a history of hay fever and skin tests (mite, mold, and pollen tested). Both 48-hour patch tests (Finn chambers) and scratch tests were performed according to International Contact Dermatitis Research Group (ICDRG) recommendations. For 2 of the patients, positive (urticarial) scratch-test reactions to p-Phenylenediamine mix (black rubber mix) were reported.

Katsarou et al. (1999) studied the incidence of immediate contact reactions (wheal and flare) in 664 patients (308 males, 356 females; ages: 7 to 74 years) with suspected contact dermatitis. In patch tests, Finn chambers were applied to the upper back for 30 minutes and then partially removed. Sites were reexamined and reactions evaluated after 5 minutes. Positive reactions were considered those with well-distinguished erythema at the test site. After recording for immediate reactions, the patches were reapplied and removed at day 2; tests were evaluated for delayed sensitivity at days 2 and 4. The number of immediate reactions and their association with delayed reactions to the same allergen in 664 patients was presented. Two immediate reactions at 30 minutes and 11 delayed reactions (to p-Phenylenediamine were reported.

Skin Irritation

p-Phenylenediamine

A 50% aqueous slurry of p-Phenylenediamine was applied to the skin of 6 subjects for 24 hours under occlusive conditions. Skin reaction was assessed at 24 and 72 hours for erythema (0 to 4) and edema (0 to 4). Erythema and edema values were added and averaged for the 2 skin readings to yield a PII. The PII for the p-Phenylenediamine on human skin was 0.8 (maximum possible total of 8) (Davies et al. 1972).

Skin Sensitization - Predictive Tests

Table 12 summarizes predicitve and provocative skin sensitization data.

p-Phenylenediamine

Kligman (1966) applied five 48-hour induction patches

containing 1 ml of 10% p-Phenylenediamine in petrolatum, with 24-hour rest periods between the patches, to the forearms or calves of 24 "mostly black" volunteers from a prison. The challenge application was a 48-hour occluded patch, containing 0.4 ml of 0.5% p-Phenylenediamine in petrolatum, on the back. All 24 of the

Table 12. Results of predictive and provocative patch tests with p-Phenylenediamine.

Material Tested	Method	Subjects	Results	Reference
		Predictive Tests		
p-Phenylenediamine at 10%	patch test (maximization procedure)	24 subjects	sensitization in all subjects	Kligman 1966
p-Phenylenediamine at 0.01, 0.1, or 1%	repeated insult patch test (RIPT)	Groups of 97, 98, and 88 subjects	sensitization: 7 of 97 (0.01% at induction; 0.01% at challenge); 11 of 98 (0.1% at induction; 1% at challenge); 47 of 88 (1% at induction; 1% at challenge)	Marzulli and Maibach 1974
p-Phenylenediamine at 2%	patch test (maximization procedure)	34 subjects	15 of 34 with sensitization reactions	Epstein and Taylor 1979
Hair dye composition containing 2% p-Phenylenediamine	patch test. Semi-occlusive patch application and initial 1-h challenge (dye, 100%), followed by 9 24-h induction patches with 10% (v/v) aqueous solution of dye over 22-day period. 1-h final challenge patch with dye (100%)	22 subjects	20 negative reactions and 5 reactions (slight dermatitis) at initial challenge. 19 negative reactions and 6 reactions (slight dermatitis) at induction. Final challenge: 8 reactions (no dermatitis), 7 reactions (slight dermatitis), and 7 reactions (significant dermatitis)	Hill Top Research 1979
Hair dye containing 0.4% p-Phenylenediamine	RIPT (nonocclusive patches)	206 subjects	no positive reactions at induction or challenge	Derma-Test Laboratories (DTL) 1982a
Hair dye containing 0.49% p-Phenylenediamine	RIPT (nonocclusive patches)	206 subjects	no positive reactions at induction or challenge	DTL 1982b
Hair dye containing 0.596% p-Phenylenediamine	RIPT (nonocclusive patches)	206 subjects	no positive reactions at induction or challenge	DTL 1982c
Hair dye containing 2.144% p-Phenylenediamine	RIPT	206 subjects	no positive reactions at induction or challenge	DTL 1982d
Hair dye composites containing up to 3.5% p-Phenylenediamine	patch test	3500 (total number of individual hair dye applications was 116,647 from 1975 to 1983)	205 positive reactions in 163 females. Most of the subjects reporting reactions had later applications of hair dyes. Only 8 reactions in 4 subjects identified as allergic responses to the products	CTFA 1983
p-Phenylenediamine at 1% and 0.05 mg/cm^2 in petrolatum	TRUE test (ready-to-apply patch test system - 0.05 mg/cm^2 tested) and Finn chamber (1% tested) patch test techniques; 48-h application and reactions scored at day 4; reactions with score of ≥1+ classified as positive	13 subjects.	4 of 13 positive in both tests; 1 of 13 positive in TRUE test only; 8 of 13 positive in Finn chamber test only	Goh 1992
p-Phenylenediamine at 1% in petrolatum	patch test. Occlusive patches applied 3 times per week for 3 weeks. 48-h challenge patch	98 healthy volunteers.	3 of 98 with grade 1 or grade 2 challenge reaction at 96 h.	Basketter et al. 2006

Table 12 (continued). Results of predictive and provocative patch tests with p-Phenylenediamine.

Material Tested	Method	Subjects	Results	Reference
p-Phenylenediamine at 1% in petrolatum	patch test. 48-h occlusive patch application	2171 healthy adults with no history of hair dye allergy and who had not dyed their hair during past 2 years divided into 3 groups: (1) exposed to hair colorant containing p-Phenylenediamine; (2) exposed to permanent hair dye, or (3) unexposed for duration of study.	Positive reactions: (1) 80 of 1107; (2) 7 of 548; (3) 2 of 516	Basketter et al. 2006
		Provocative Tests		
p-Phenylenediamine at 2% in petrolatum	patch tests on thigh or back	5558 Scandinavian patients (over 2 year span) from 6 clinics	4.5% reacted positively	Magnusson et al. 1968
p-Phenylenediamine at 1% in petrolatum	patch tests	4,825 European patients	4.9% (237) reacted positively	Fregert et al. 1969
p-Phenylenediamine at 1%	Al-test patches	281 housewives with contact dermatitis of the hands; 1000 people doing domestic work only (this includes the 281 women) (patients from 5 European clinics)	5% of both populations had positive reactions to p-Phenylenediamine	Calnan et al. 1970
p-Phenylenediamine at 1% in petrolatum	patch tests	184 men and 116 women suspected of having contact dermatitis (from Belgium)	13.0% (24) of the men and 4.3% (5) of the women were positive; 9.7% of all of the subjects were positive	Oleffe et al. 1972
p-Phenylenediamine at 2% in petrolatum	patch tests	540 patients (in U.S.A. during 1968 to 1970), the majority with contact dermatitis; reactions scored on a 1+ to 4+ scale	13.5% reacted to p-Phenylenediamine; 12, 24, 26, and 11 patients with 1+, 2+, 3+, and 4+ reactions, respectively	Baer et al. 1973
p-Phenylenediamine at 2% in petrolatum	patch tests	229 patients (in U.S.A. during 1968 to 1970) suspected of having contact dermatitis due to shoes or rubber. Reactions were scored on a 1+ to 4+ scale	7.0% reacted to p-Phenylenediamine; 1, 3, 12, and 0 patients with 1+, 2+, 3+, and 4+ reactions, respectively	
p-Phenylenediamine at 1% in petrolatum	Al-test patches; removed after 48 hours and reactions recorded at 48, 72, and/or 96 hours	1200 patients from private and outpatient clinics in North America (from January 1, 1971 to June 30, 1972)	8% (98) reacted positively	Rudner et al. 1973
p-Phenylenediamine at 1% in petrolatum	Al-test patches; removed after 48 hours and reactions recorded at 48, 72, and/or 96 hours	3041 patients from private and outpatient clinics in North America (from July 1, 1972 to June 30, 1974)	6.1% reacted positively	Rudner et al. 1975
p-Phenylenediamine at 1% in water	closed patch test for nonsensitized subjects and open patch test for sensitized subjects	32 hairdressers who had never suffered from allergic contact dermatitis due to p-Phenylenediamine and 7 hairdressers who had strongly positive reactions to p-Phenylenediamine (from Japan)	0/32 and 6/7 positive reactions for the nonsensitized and sensitized hairdressers, respectively	Morikawa et al. 1976

Table 12 (continued). Results of predictive and provocative patch tests with p-Phenylenediamine.

Material Tested	Method	Subjects	Results	Reference
p-Phenylenediamine at 1%	patch tests	2363 tested (from 1978-1979) in the U.S.A.	7% (157) positive reactions	Morikawa et al. 1976
p-Phenylenediamine at 1%	patch tests	2094 tested (from 1979-1980) in the U.S.A.	6% (136) positive reactions	Morikawa et al. 1976
p-Phenylenediamine at 1% in petrolatum	Al-test patches on the back; results read at 48 and 72 hours	155 Japanese hospital patients, mainly outpatients	22.58% (35) were positive	Fujiwara et al. 1976
p-Phenylenediamine at 2% in petroalatum	Japanese-made patches on the back. Results read at 48 and 72 hours	196 Japanese hospital patients, mainly outpatients (from September of 1973 to August of 1975)	28.57% (55) were positive	Fujiwara et al. 1976
p-Phenylenediamine - concentration not stated	patch tests on back removed at Day 2 and read at Days 2 and 7. (Other chemicals tested simultaneously). Positives were reapplied on Day 7 and removed and read on Day 9	35 patients (from Canada)	17% (6) positive test reactions on Day 2 and 5 or 14% positive test reactions on Day 9	Mitchell 1977
p-Phenylenediamine at 2% in petrolatum	patch tests on the hand	250 hospital patients and 250 private patients (spans 3 years in France)	6.8% (34) of the subjects gave positive responses to p-Phenylenediamine, with little difference between hospital and private patients	Calas et al. 1978
p-Phenylenediamine at 2% in petrolatum	occluded patches applied to the back. Patches removed at 48 hours and read at 48 and 96 hours	536 patients (tested in 1976, from Brazil)	1.1% reacted positively	Moriearty et al. 1978
p-Phenylenediamine at 1% in petrolatum	Al-test patches; reactions read 48 and 96 hours after patch application	2806 patients (in Spain during 1977), from contact dermatitis sections of hospitals or an occupational dermatitis center	9.90% (278) were positive to p-Phenylenediamine; 14.02, 10.43, and 8.63 % were masons, metallurgists, and housewives, respectively	Camarasa 1979
p-Phenylenediamine (in experimental hair dye) mixed with hydrogen peroxide (5 ml)	Open patch tests	6 subjects (open patch test)	positive reactions in 4 of 6 subjects	Epstein and Taylor 1979
p-Phenylenediamine (in experimental hair dye) mixed with hydrogen peroxide (5 ml)	Open and closed patch tests	7 subjects (open patch test); 12 subjects (closed patch test)	positive reactions: 4 of 7 subjects (open patches) and all 12 subjects (closed patches)	Epstein and Taylor 1979
p-Phenylenediamine (in experimental hair dye) mixed with hydrogen peroxide (15 ml)	Open and closed patch tests	6 subjects (open patch test); 12 subjects (closed patch test)	positive reactions: 4 of 6 subjects (open patches) and all 12 subjects (closed patches)	Epstein and Taylor 1979
p-Phenylenediamine at 1% in petrolatum	patch tests on the back; readings after 48 and 72 hours	53 denture-wearing patients with "burning mouth syndrome" (from Denmark)	1 positive reaction (erythema and infiltration with papules or vesicles)	Kaaber et al. 1979
p-Phenylenediamine at 1% in petrolatum	patches applied, removed at Day 2; reactions read at Day 2 and Day 4	225 men and 175 women with hand eczema (from Belgium)	9.3% (21) of the men and 14 or 8% of the women reacted positively	Lachapelle and Tennstedt 1979
p-Phenylenediamine at 1% in petrolatum	patch tests	108 patients (spans 4.5 years in Italy) with contact dermatitis of the feet correlated clinically with shoe contact	24.8% (41) of the subjects were positive to p-Phenylenediamine	Angelini et al. 1980

Table 12 (continued). Results of predictive and provocative patch tests with p-Phenylenediamine.

Material Tested	Method	Subjects	Results	Reference
p-Phenylenediamine at 1% in petrolatum	patch tests	13 eczema patients allergic to a brown stocking dye (from Finland)	11 patients were positive	Kousa and Soini 1980
p-Phenylenediamine at 1% in petrolatum	patch tests	362 Finnish eczema patients (from March 1 to September 30, 1979)	2.5% (9) of the subjects were positive to p-Phenylenediamine	Kousa and Soini 1980
p-Phenylenediamine at 1% in petrolatum	patch tests	66 hairdressers with eczema (1973-1981; from Canada)	45% (30) were positive	Lynde and Mitchell 1982
p-Phenylenediamine at 1% in petrolatum	patch tests on the back for 48 hours; read at 48 and 96 hours	200 Canadian hospital clinic patients with eczematous dermatitis (1977-1979)	30% were positve	Nethercott 1982
p-Phenylenediamine at 1% in petrolatum	patch test on the upper back for 48 hours; read at 48 and/or 72 hours	149 patients from private practices and clinics in the U.S.A. with cosmetic-related contact dermatitis (1977-1980)	16% (24) were positive	NACDG 1982
p-Phenylenediamine at 1% in petrolatum	patch test (20-minute test)	129 patients	3 patients with positive reactions; all of whom also developed urticaria.	Temesvari 1984
p-Phenylenediamine at 1% in petrolatum	patch tests: open -filter paper disc (reactions scored at 45 min) and Finn chambers (48-h application; reactions scored at 48 h and 96 h	50 volunteers: 19 controls, 15 (with eczematous dermatitis), and 16 (with cosmetic sensitivity)	no contact urticaria in the open patch test; 12 positive reactions using Finn chambers	Emmons and Marks 1985
p-Phenylenediamine - concentration not stated	patch test: 48-h application ; reactions scored at 48 h and 72 h	13,216 dermatitis patients	41 cutaneous reactions	Adams and Maibach 1985
p-Phenylenediamine - concentration not stated	patch test	25 patients with hand eczema	25 positive reactions	Cronin 1985
p-Phenylenediamine mixture at 1% in petrolatum	patch test	539 dermatitis patients	36 positive reactions	Correia and Brandao 1986
p-Phenylenediamine - concentration not stated	patch test	25 patients with leg ulcers	2 positive reactions	Kokelj and Cantarutti 1986
p-Phenylenediamine - concentration not stated	patch test	5202 patients	10.3% of patients with positive reaction	Broeckx et al. 1987
p-Phenylenediamine at 1% in vaseline	patch test	190 children	67 positive patch test reactions. Dermatitis induced in 7% of patients with positive reactions	De la Cuadra Oyanguren et al. 1989
p-Phenylenediamine at 1% in petrolatum	patch test: 220 tested in 1975; 240 tested in 1987	460 patients with contact sensitization	10.52% with positive reactions in 1975; 8.52% with positive reactions in 1987	Stransky and Krasteva 1989
p-Phenylenediamine at 1% in petrolatum	patch test (Finn chamber): 48 h or 72 h application	1138 dermatitis patients	79 allergic reactions; 2 irritant reactions	Storrs et al. 1989
p-Phenylenediamine - concentration not stated	patch test	19 patients with eczema and allergic reactions to disperse dyes	6 positive reactions	Balato et al. 1990
p-Phenylenediamine - concentration not stated	patch test (Finn chamber): 48-h application; reactions scored at 72 h and 96 h	204 dermatitis patients	18 positive reactions	Fan and Zhao 1990

Table 12 (continued). Results of predictive and provocative patch tests with p-Phenylenediamine.

Material Tested	Method	Subjects	Results	Reference
p-Phenylenediamine - concentration not stated	patch test (Finn chamber): 48 h patch test; reactions scored at 48 h, 72 h, and 96 h.	921 patients with differential diagnosis, including contact dermatitis	5.4% sensitization rate	Bruckner-Tuderman et al. 1992
p-Phenylenediamine at 0.5% in petrolatum	patch test; reactions scored at 2 and 3 days	261 dermatitis patients	19 positive reactions	Guerra et al. 1992a
p-Phenylenediamine at 0.5% in petrolatum	patch test (Finn chamber):	1285 patients	82 non-negative reactions	Brasch et al. 1994
p-Phenylenediamine - concentration not stated	patch test	79 patients (96% with eczema)	2 positive, relevant reactions	Berne et al. 1996
p-Phenylenediamine at 3.75%	patch test	80 chronic hemodialysis patients	14 patients with patch test reactions to various substances, 3 of whom had a positive reaction to 3.75% p-Phenylenediamine	Gonzalo et al. 1997
p-Phenylenediamine - concentration not stated	patch test	84 dermatitis patients (azo-dye positive)	40 positive reactions	Seidenari et al. 1997
p-Phenylenediamine at 1% in petrolatum	patch test (Finn chamber): 48-h application	83 children with dermatitis	2 positive reactions	Shah et al. 1997
p-Phenylenediamine at 0.01% to 1% in petrolatum	patch test; 15, 30, 120, and 165 min applications	16 patients: 7 patch tested with 1% for up to 120 min; 9 patch tested with 0.01%, 0.1%, 0.3%, and 1% for up to 165 min	Following 120-min exposure, 11 of 16 reacted to1% p-Phenylenediamine and 2 of 9 reacted to 0.01% p-Phenylenediamine. Most reactions were + and ++.	McFadden et al. 1998
p-Phenylenediamine at 1%	patch test (Finn chamber): 48-h application; reactions scored at 48 h to 72 h and between 72 h and 168 h	111 dermatitis patients	6.8% with allergic reaction	Marks et al. 1998a
p-Phenylenediamine at 1%	patch test (Finn chamber): 48-h application; reactions scored at 48 h to 72 h and between 72 h and 168 h.	5831 dermatitis patients	4.9% with allergic reaction; 0.1% with irritant reaction	Marks et al. 1998b
p-Phenylenediamine - concentration not stated	patch test data collected over 4-month period (retrospective survey)	475 patients with contact allergy	33 positive reactions	Goossens et al. 1999
p-Phenylenediamine - concentration not stated	patch test: 48-h application. Study to determine frequency of "lost", "found", and "persistent" reactions to standard screening tray	587 patients	10 positive reactions	Dickel et al. 2000
p-Phenylenediamine - concentration not stated	patch test (Finn chamber): 48-h application; reactions scored at 72 h and between 96 and 192 h	991 patients	statistically significant difference (P = 0.00599) in sensitization rate in black patients (10.6%) when compared to white patients (4.5%)	Dickel et al. 2001
p-Phenylenediamine - concentration not stated	patch test	678 patients	with and without simultaneous testing with para-aminoazobenzene, 4.3% and 3.1% of the patients, respectively, reacted to p-Phenylenediamine	Devos and Van Der Valk 2001
p-Phenylenediamine - concentration not stated	patch test (Finn chamber): reactions scored at 2 and 3 days	105 hand dermatitis patients; 361 non-hand allergic contact dermatitis patients	positivity rates in hand dermatitis group and control group were 14.3% and 14.1%, respectively	Li and Wang 2002
p-Phenylenediamine - concentration not stated	patch test: reactions scored at 72 h	638 patients	14.1% with positive reactions	Uter et al. 2002

Table 12 (continued). Results of predictive and provocative patch tests with p-Phenylenediamine.

Material Tested	Method	Subjects	Results	Reference
Hair coloring product containing 1.8% p-Phenylenediamine	open test (test recommended 48 h prior to hair dye application): 48-h application, no rinsing, and reactions scored at 1 h post-application and on days 2 and 4	30 contact dermatitis patients with positive patch test reactions to p-Phenylenediamine; 30 sex- and age-matched p-Phenylenediamine-negative subjects with no history of adverse reactions to hair coloring products	allergic reactions in all p-Phenylenediamine positive patients; no reactions in p-Phenylenediamine-negative subjects	Krasteva et al. 2002
p-Phenylenediamine at 1% in petrolatum	patch test (Finn chamber): 48-h application; reactions scored at 48 h to 72 h and between 72 h and 168 h	9624 patients	sensitization rates: 1992-1994 (blacks: 7.8%; whites 5.8%), 1994-1996 (blacks: 13.5%; whites: 5.8%, P< 0.05 - significant difference), and 1996-1998 (blacks: 10.3%; whites: 5.3%, P < 0.05)	Deleo et al. 2002
p-Phenylenediamine in petrolatum - concentration not stated	patch test (Finn chamber): 48-h application; reactions scored at day 3 and between days 4 and 8	991 patients (877 whites; 114 blacks)	statistically significant difference (P = 0.00599) in sensitization rate in black patients (rate = 10.6 %) when compared to white patients (rate = 4.5%)	Dickel et al. 2001
p-Phenylenediamine - concentration not stated	patch test	191 children	sensitization rate = 6%	Lewis et al. 2004
p-Phenylenediamine - concentration not stated	patch test (Finn chamber); reactions scored on day 2 and on day 3 or day 4	27 patients with lichen simplex chronicus (LSC) who dyed their hair regularly	11 patients with positive reactions	Chey et al. 2004
p-Phenylenediamine - concentration not stated	patch test (Finn chamber); reactions scored on day 2 and on day 3 or day 4	560 dermatitis patients with concurrent LSC and/or other neurodermatitis	63 weakly positive reactions	Chey et al. 2004
p-Phenylenediamine at 1% in petrolatum	patch test	13,300 dermatitis patients	449 positive reactions from years 1990 to 2000	Dawe et al. 2004
p-Phenylenediamine at 1% in petrolatum	patch test (Finn chamber): 48-h application; reactions scored at 48 h to 72 h and between 72 h and 168 h post-application	4,913 patients	4.8% of patients with positive reactions; 0.2% with irritant reactions	Pratt et al. 2004
p-Phenylenediamine at 1% in petrolatum	patch test (Finn chamber): 48-h application; reactions scored at 48 h to 72 h and at 96 h to 168 h post-application	1,320 patients	4.9% of patients with positive reactions	Wetter et al. 2005
p-Phenylenediamine - concentration not stated	patch test	378 eczema patients (73 suspected of having cosmetic allergic contact dermatitis [CACD]; 37 with confirmed CACD	positive patch test reactions in patients suspected of having CACD and in those with confirmed CACD were 5.5% and 8.1%, respectively	Wang et al. 2005
Oxidative hair dye products containing p-Phenylenediamine at 0.1, 0.5, 1.0, or 1.5%	open patch test (recommended 48 h prior to hair coloring)	34 subjects with allergic reactions to p-Phenylenediamine; 49 non-allergic subjects	cumulative allergic reactions as follows: 0.1% p-Phenylenediamine (27 of 34 subjects), 0.5% p-Phenylenediamine (30 of 34), 1.0% p-Phenylenediamine (33 of 34), or 1.5% p-Phenylenediamine (34 of 34), corresponding to 79, 88, 97, or 100% of the study subjects, respectively	Krasteva et al. 2005

Table 12 (continued). Results of predictive and provocative patch tests with p-Phenylenediamine.

Material Tested	Method	Subjects	Results	Reference
p-Phenylenediamine - concentration not stated	patch test	9948 dermatitis patients	sensitization frequency of 4.2%	Oppel and Schnuch 2006
p-Phenylenediamine - concentration not stated (impurities: 0.1% Bandrowski's base and traces of 4,4'-azodianiline)	patch test (Finn chamber)	159 dermatitis patients	8 positive reactions. 1 patient with positive reaction to 1% Bandrowski's base	White et al. 2006
p-Phenylenediamine at 0.01%, 0.1%, and 1% (impurities: 0.1% Bandrowski's base and traces of 4,4'-azodianiline)	patch test (Finn chamber)	6 dermatitis patients at one clinic; 29 dermatitis at another (all patients [both clinics] patch test positive to p-Phenylenediamine); 642 normal volunteers	p-Phenylenediamine positive reactions in dermatitis patients: 1 of 6 (0.01% concentration); 3 of 6 (0.1%); 5 of 6 (1%); 26 of 29; normal volunteers: 14 of 642; 5 of 14 tested were positive to 1% Bandrowski's base	White et al. 2006
p-Phenylenediamine at 1% in petrolatum	patch test	25 patients	24 positive reactions	Uter et al. 2006
p-Phenylenediamine at 1% in petrolatum and 2 complete permanent black hair dyes, undiluted	patch test, group 1 (1% in petrolatum, Finn chamber): 48-h application; reactions scored at day 2. Patch test, groups 2 and 3 (2 hair dyes, respectively): 30- min, 1- h, and 24-h applications per group; reactions scored at days 1, 2, and 3 post-removal	3 groups of 15 volunteers (all allergic to p-Phenylenediamine) with positivie reactions at 2 days post-removal.	24-h application of either hair dye was the only duration sufficient to yield positive reactions in all subjects in the 2 hair dye treatment groups; frequency of hair dye positive reactions (after 24 h) comparable to that observed following 48-h exposure to 1% p-Phenylenediamine in petrolatum	Jowsey et al. 2006
p-Phenylenediamine at 1% and serial dilution (1 to 10,000 ppm) in petrolatum	patch test (Finn chamber): 48-h application; reactions scored on days 3 and 7	15 patients (allergic to p-Phenylenediamine)	14 patients with at least a weakly positive reaction to 1% p-Phenylenediamine; threshold value for 10% of patients tested, based on + reaction or greater on back, was 38 ppm	Søsted et al. 2006a
p-Phenylenediamine - concentration not stated	patch test (Finn chamber: 48-h application; reactions scored at 48 h and 96 h	500 children with dermatitis	Sensitization rate of 8%	Clayton et al. 2006
p-Phenylenediamine at 1% in petrolatum	occlusive patch tests: 24-h and 48-h applications. Reactions scored on days 1-3 (24-h test) and on days 2-3 (48-h test). Late readings made on days 7, 14, and 21 post-application	data on 1428 patients were evaluable; two-thirds 48 h and one-third 24 h readings	21 of 1428 with positive reactions; with exception of 1 reaction, all late reactions observed in patients in 48-h test	Hillen et al. 2006
p-Phenylenedaimine at 1% in petrolatum	patch test	1,222 dermatitis patients	144 (11.8%) wih positive reactions	Hillen et al. 2007
p-Phenylenediamine - concentration not stated	patch test; reactions scored at 2/3 days and 4/5 days	6,177 dermatitis patients	positive reactions ranged from 3.8% in 1989 to 7.1% in 2004	Patel et al. 2007
0.5% p-Phenylenediamine HCl; 1% p-Phenylenediamine base	patch test: 48-h application; reactions scored 20 to 60 min after patch removal and 24 and 48 h after first reading. p-Phenylenediamine HCl tested from 1984 to 1988; p-Phenylenediamine base tested from 1989 to 1993	42,839 dermatitis patients	1481 positive reactions to p-Phenylenediamine	Sertoli et al. 1999
0.5% p-Phenylenediamine HCl; 1% p-Phenylenediamine free base	patch test. p-Phenylenediamine Dichloride tested between 1985 and 1988; p-Phenylenediamine was tested for remaining years, up to 1998	26,706 dermatitis patients	667 positive reations to p-Phenylenediamine	Armstrong et al. 1999

Table 12 (continued). Results of predictive and provocative patch tests with p-Phenylenediamine.

Material Tested	Method	Subjects	Results	Reference
p-Phenylenediamine HCl at 0.5%	patch test	80 patients	27 positive reactions	Picardo et al. 1990
p-Phenylenediamine HCl - concentration not given	patch test (Finn chamber): 48-h application; reactions scored at 48 h, 72 h, and 96 h	107 dermatitis patients	17 positive reactions	Zhao and Fan 1991
p-Phenylenediamine HCl at 0.5%	patch test (Finn chamber; reactions scored at days 2 and 4	437 patients: 256 (contact dermatitis), 109 (endogenous eczema), and 72 (unclassified eczema)	1 of 256 with allergic reaction; no positive reactions in the 2 eczema groups	Lee and Lam 1996

subjects were sensitized to p-Phenylenediamine.

Marzulli and Maibach (1974) reported a study in which, 10 occluded patches, containing 0.5 g of a 0.01, 0.1, or 1.0% solution of p-Phenylenediamine in petrolatum, were applied to the skin of male subjects for 48 or 72 h. This was followed by a 2-week rest period. The challenge was a 72-hour patch with a nonirritant concentration.

The 0.01% p-Phenylenediamine induction application was followed by a 0.01 percent challenge patch. Seven of 97, or 7.2% of the men, responded positively to the challenge.

The 0.1% p-Phenylenediamine induction application was followed by a 1 .0 percent challenge patch, and 11 of 98, or 11.2% of the men, responded positively.

The 1.0 percent p-Phenylenediamine induction application was followed by a 1.0% challenge application.

Forty-seven of 88, or 53.4% of the men, responded positively. The authors suggested an increase in the incidence of sensitization with higher concentrations of p-Phenylenediamine used in induction applications (Marzulli and Maibach 1974).

Epstein and Taylor (1979) used a 2% aqueous solution of p-Phenylenediamine for induction applications and challenge patches in a maximization test with 34 male volunteers from a correctional facility as subjects. Fifteen of the 34, or 44% of the subjects, were sensitized to p-Phenylenediamine.

Hilltop Research (1979) entered 25 panelists into a patch test study with a dye composition containing 2% p-Phenylenediamine. Three panelists quit the study before the final challenge application. Semiocclusive patches containing 0.3 ml of the test material were placed on the arms. There was an initial 1-hour challenge patch with 100% of the dye followed by 9 24-hour induction patches with a 10% (v/v) aqueous solution of the dye over a 22-day period. The final challenge patch was a 1-hour patch with 100% of the dye on both the original site and a new site. Reactions were scored on a scale of 0 to 7.

At the initial challenge there were 20 negative reactions (scores of 0 to 1) and 5 reactions of slight dermatitis (scores of 2 to 3). Nineteen of the panelists had negative reactions during the induction period, and 6 had reactions of slight dermatitis. There were 8 reactions of no dermatitis, 7 reactions of slight dermatitis, and 7 reactions of significant dermatitis (4 to 7) at the final challenge patching (Hilltop Research 1979).

Derma-Test Laboratories (DTL) conducted a repeated insult patch test with a hair dye containing 0.039% 4-nitro-o-phenylenediamine (4NOPD) and 0.4% p-Phenylenediamine (DTL 1982a). Two hundred six subjects were enrolled in and completed the study. The dye was mixed with an equal volume of oxidizer, and each nonocclusive patch contained 0.1 ml/cm^2 of the dye and oxidizer mixture. Ten 48- to 72-hour consecutive patch applications were made on the backs of the subjects, and reactions were read after removal of each patch. These induction patches were followed by an 11-day rest period. A 48-hour nonocclusive challenge patch was applied to a previously unexposed site on the back of each subject, and the reaction was read at removal and at 15 minutes and at 24 hours later.

There were 41 doubtful reactions (very mild erythema, barely exceeding that of untreated skin) during induction. There were no positive reactions at any induction or challenge reading. The researchers stated that there was no evidence to indicate that the hair dye and oxidizer test product caused either irritation or sensitization (DTL 1982a).

A repeated insult patch test was conducted with a hair dye containing 0.49% p-Phenylenediamine and 0.027% 4NOPD on the same 206 subjects and following the same procedure (DTL 1982b). There were no positive reactions at any induction or challenge reading. The researchers stated that their data provided no evidence that the hair dye and oxidizer test product caused either irritation or sensitization.

A repeated insult patch test was conducted with a hair dye containing 0.596% p-Phenylenediamine and 0.049% 4NOPD on the same 206 subjects and following the same procedure (DTL 1982c). There were no positive reactions at any induction or challenge reading. The researchers stated that their data provided no evidence to indicate that the hair dye and oxidizer test product caused either irritation or sensitization.

A repeated insult patch test was conducted with a hair dye containing 2.144% p-Phenylenediamine on the same 206 subjects and following the same procedure. (DTL 1982d) There were no positive reactions at any induction or challenge reading. The researchers stated that their data provided no evidence that the hair dye and oxidizer test product caused either irritation or sensitization.

CTFA (1983) reported a study in which a hair dye company performed preliminary patch tests on approximately 3500 prospective models in New York, NY (total number of individual hair dye applications was 116,647) with hair dye composites containing up to the maximum amount of p-Phenylenediamine used in the product line (3.5%) over the period 1975 to 1983. (Virtually all of the dyes contained p-Phenylenediamine.) Two hundred five positive reactions were observed in 163 women. Most of the women who reported reactions had later applications of hair dyes. Only 8 reactions on 4 women were identified as allergic responses to the products.

Goh (1992) patch tested 13 subjects (mean age = 34.5 years) using TRUE test (ready-to-apply patch test system) and Finn chamber patch test techniques. Test strips were applied symetrically on upper back, and removed after two days. Reactions were scored at day 4. In the TRUE test, p-Phenylenediamine was tested at a concentration of 0.05 mg/cm^2. In the Finn chamber test, p-Phenylenediamine was tested at a concentration of 1% in petrolatum. Reactions with a score of $\geq 1^+$ were classified as positive. Four of the 13 subjects had positive reactions in both tests. One of the 13 subjects had a positive reaction in the TRUE test only. Eight subjects had positive reactions in the Finn chamber test only.

Basketter et al. (2006) conducted a human repeated insult patch test in which 98 healthy volunteers were patch tested with 1% p-Phenylenediamine in petrolatum. Occlusive patches were applied to the upper arm (deltoid region) for 5 minutes three times per week for a total of three weeks. During the challenge phase, occlusive patches containing 1% p-Phenylenediamine (in petrolatum) weere applied for 48 hours, and reactions were scored at 30 minutes post-removal and at 96 hours post-application. Three of the 98 volunteers (3%) had a grade 1 or grade 2 challenge reaction at 96 hours.

In a second experiment, 2171 healthy adults with no history of hair dye allergy who had not dyed their hair during the past 2 years were divided into the following groups: group 1 (1107 subjects: exposure to p-Phenylenediamine in hair colorant formulation base + peroxide activating system [final concentration on head = 0.48% p-Phenylenediamine] 5 min per day for 4 consecutive days, then once per week for remainder of 6- month exposure period), group 2 (548 subjects: exposure to permanent hair dye containing p-Phenylenediamine [final concentration on head = 1.5% p-Phenylenediamine] once per month; ~30 to 40 min per exposure, 6 exposures total), and group 3 (516 subjects: unexposed for duration of the study). A 48-hour occlusive patch test (1% p-Phenylenediamine in petrolatum) was conducted at the end of the experiment. Sites were scored at 1 hour post-removal according to the ICDRG grading scale. The highest response occurred in group 1 (80 of 1107 [7.2%] with positive reactions). In group 2, 7 of 548 volunteers (1.3%) had positive reactions. The incidence of positive reactions in group 3 was 0.4% (2 of 516 volunteers) (Basketter et al. 2006).

Skin Sensitization - Provocative Tests

The 30 provocative tests reported through 1982 are summarized in Table 12, but not in the text that follows. Generally, these patch tests were performed with 1 or 2% p-Phenylenediamine in petrolatum, and 1.1 to 84.6% of the patients were positive for p-Phenylenediamine.

The 48 studies after 1982 are summarized in Table 12 and discussed below.

p-Phenylenediamine

Temesvari (1984) performed 20-minute patch tests on 129 patients. Three of the patients had positive reactions to 1% p-Phenylenediamine in petrolatum. Two of the patients (28 and 32 years old) had urticaria that developed while dyeing their hair. Initially, the urticaria was localized on hairy skin, but later spread over the body. The 3rd patient (52 years old) used black cotton thread in her profession and had painful palms (with erythema and edema), and urticaria developed over her body. Patch testing with p-Phenylenediamine also provoked 1 urticarial reaction.

Adams and Maibach (1985) patch tested 13,216 patients with contact dermatitis over a 64-month period. Patch tests applied to upper back for 48 hours and reactions were scored at 48 and 72 hours. Forty-one cutaneous reactions to p-Phenylenediamine were reported.

In a study by Cronin (1985), one of 25 women (ages not stated) with occupational hand eczema had a positive patch test reaction to p-Phenylenediamine.

Emmons and Marks (1985) patch tested 50 volunteers (19 males, 31 females), comprised of 19 controls, 15 with eczematous dermatitis, and 16 with cosmetic sensitivity. In open testing, 1% p-Phenylenediamine in petrolatum (several milliliters) was smeared on to the skin, and the application

site was covered with a filter paper disc. Reactions were scored at 45 minutes. Patches (Finn chambers) containing p-Phenylenediamine in petrolatum were also applied to the back for 48 hours; reactions were scored at 48 and 96 hours.

Results of open patch testing indicated no contact urticaria. In patch tests using Finn chambers, p-Phenylenediamine produced 12 positive reactions, the majority of which were not clinically relevant. There was no correlation between a history of cosmetic sensitivity determined via questionnnaire and patch test results (Emmons and Marks 1985).

Correia and Brandao (1986) patch tested 539 dermatitis patients (50% between 30 and 50 years old) with a 1% p-Phenylenediamine mixture (in petrolatum) over a period of 12 years. Thirty-six patients had positive patch test reactions to p-Phenylenediamine.

Kokelj and Cantarutti (1986) patch-tested 25 patients (120 males, 15 females; average age: 69 years) with leg ulcers. Two of the patients had positive patch test (sensitization) reactions to p-Phenylenediamine.

Broeckx et al. (1987) patch tested 5202 patients (3330 women, 1872 men; ages not stated) in a study of cosmetic intolerance. The authors considered allergy to hair dyes to be important, in that 10.3% of the patients reacted to p-Phenylenediamine.

De la Cuadra Oyanguren et al. (1989) conducted a retrospective study involving 190 children (ages: 2 to 14) with dermatitis. Of the 190 children, 67 (35%) had positive patch test reactions. p-Phenylenediamine (1% in vaseline) induced dermatitis in 7% of the patients with positive patch test reactions.

Between January 1, 1984 and May 1, 1985, Storrs et al. (1989) patch tested 1138 patients (mean age = 42.9 years) suspected of having allergic contact dermatitis with 1% p-Phenylenediamine in petrolatum using Finn chambers. Patches remained in place for 48 or 72 hours. Seventy-nine allergic reactions and 2 irritant reactions were reported. A relevance value of 59% was assigned to this allergen.

Stransky and Krasteva (1989) patch tested 460 patients (21 to 40 years old; mostly male) with contact sensitization. Two-hundred twenty patients were patch tested in 1975, and 240 patients were patch-tested in 1987. The percentages of patients with positive reactions to p-Phenylenediamine (1% in white petrolatum) were 10.52% in 1975 and 8.52% in 1987.

Fan and Zhao (1990) patch tested 204 patients (69 males and 135 females; ages 15 to 59 years) suspected of having allergic contact dermatitis between March of 1988 and March of 1989. Patches (Finn chambers) remained in place for 48 hours and reactions were scored at 72 and 96 hours. Eighteen patients had positive reactions to p-Phenylenediamine.

Balato et al. (1990) patch tested 19 patients (6 males, 13 females) with various eczemas and allergic reactions to disperse dyes. Six of the 19 patients had positive reactions to p-Phenylenediamine. The 6 also reacted to Disperse Orange 3. The authors suggested that the reactions to Disperse Orange 3 were probably due to cross-sensitivity to p-Phenylenediamine.

Bruckner-Tuderman et al. (1992) patch tested 921 patients (with differential diagnoses, including contact dermatitis). Finn chambers were applied for 48 hours and reactions were scored at 48, 72, and 96 hours. Only ++ reactions with infiltration, erythema, and scattered papulovesicles, or stronger, were considered to be of an allergic nature. A 5.4% sensitization rate was associated with p-Phenylenediamine.

Brasch et al. (1994) patch tested 1285 patients (487 men, 798 women; ages not stated) with 0.5% p-Phenylenediamine free base in petrolatum. Finn chambers (on both sides of the back) remained in place for 24 or 48 hours. Of the 1285 patients, 82 had non-negative reactions to p-Phenylenediamine. Approximately 75% of the reactions that were observed on both sides of the back were allergic reactions; of these, 88% were scored as + reactions and 12% were scored as ++ or +++ reactions.

An adverse reactions report on cosmetics by Berne et al. (1996), for years 1989 to 1994, indicated that the patch testing of 79 patients (90% with eczema) resulted in 2 relevant reactions to p-Phenylenediamine.

Gonzalo et al. (1997) evaluated 80 patients (40 males, 40 females; mean age = 56 ± 17 years) who were on chronic hemodialysis (mean duration = 46 ± 50 months). The authors noted that hemodialysis implies contact with potential allergenic materials (e.g., gloves, catheters, needles, etc.). Fourteen of the 80 patients had patch test reactions to various substances. Three of 14 patients had positive patch test reactions to 3.75% p-Phenylenediamine.

Seidenari et al. (1997) investigated cross-reactions between different azo dyes and para-amino compounds in azo-dye-sensitive subjects. Of the 84 azo-dye positive subjects with hand dermatitis, 40 had positive reactions to p-Phenylenediamine (test concentration not stated).

In a study by Shah et al. (1997), 83 children (47 girls, 36 boys; mean age = 12.1 years) with dermatitis were patch tested (Finn chambers, 48-hour application) between January of 1991 and December of 1995. The mean duration of the dermatitis was 5 years; patch test reactions were scored at 2 and 4 days. Two patients had positive reactions to 1% p-Phenylenediamine in petrolatum.

McFadden et al. (1998) studied the length of the exposure time required to elicit p-Phenylenediamine allergic reactions using 16 patients (age range: 17 to 65 years). Seven patients were patch-tested with 1% p-Phenylenediamine in

petrolatum for 15 minutes, 30 minutes, and 120 minutes. The remaining 9 patients were patch tested with 1%, 0.3%, 0.1%, and 0.01% p-Phenylenediamine for 165 minutes, 30 minutes, and 120 minutes each.

Following the 120-minute exposure, 11 of 16 subjects reacted to 1% p-Phenylenediamine and 2 of 9 reacted to 0.01% p-Phenylenediamine. Following the 15-minute exposure, 6 of 16 reacted to 1% p-Phenylenediamine and 0 of 9 reacted to 0.01% p-Phenylenediamine. The majority of the reactions observed were defined as + and ++ reactions. The authors concluded that the duration of exposure required for contact allergens such as p-Phenylenediamine to elicit allergic contact dermatitis reactions may be very brief (McFadden et al. 1998) .

Marks et al. (1998a) patch tested patients suspected of having allergic contact dermatitis between July 1, 1994 and June 30, 1996. Patches (Finn chambers) remained in place for 48 hours and test sites were evaluated twice, initially at 48 to 72 hours and, again, between 72 and 168 hours after initial placement. Reactions of 1+, 2+, or 3+ were classified as positive. Of the 111 patients patch tested with 1% p-Phenylenediamine, 6.8% had an allergic reaction.

In another report by Marks et al. (1998b) over 5800 patients suspected of having allergic contact dermatitis were patch tested from July 1, 1998 to December 31, 2000. The patch test procedure was identical to that stated in the preceding paragraph. Of the 5831 patients patch tested with 1% p-Phenylenediamine, 4.9% had an allergic reaction and 0.1% had an irritant reaction.

In a study by Armstrong et al. (1999) conducted from January 1982 to December 1998, 26,706 patients (41% male, 59% female) with suspected contact dermatitis were patch tested. p-Phenylenediamine HCl (0.5% in petrolatum) was the test allergen between 1985 and 1988, while p-Phenylenediamine-free base (1% in petrolatum) was tested during the remaining years. Of the 26,706 patients patch tested, 667 (217 male, 450 female) had positive reactions (+ to +++) to p-Phenylenediamine, representing 2.5% of the total patch-test population.

Goossens et al. (1999) conducted a retrospective survey of allergic contact reactions to cosmetics. Data on 475 patients with contact allergy to cosmetic ingredients, observed during a 4-month period (January to April of 1996), were collected. A total of 33 positive reactions to p-Phenylenediamine (test concentration not stated) was reported.

Dickel et al. (2000) conducted a study to determine the frequency of "lost," "found," and "persistent" reactions to a standard screening tray by comparing initial and delayed readings of the same patch tests. The methodology involved a retrospective review of patch test reactions for 587 patients (ages not stated) who were tested between January 1, 1988 and December 31, 1991. Forty-eight-hour patch tests were performed. Results for the 587 patients were reported as follows: 81 "found" reactions (15%), 342 "persistent" reactions (62%), and 127 "lost" reactions (23%). The 3 most common "found" reactions were to neomycin sulfate (n = 19; 53%), p-Phenylenediamine (n = 10; 29%), and thiomersal (n = 10; 18%).

Devos and Van Der Valk (2001) conducted an experiment (678 patients; ages: 8 to 85 years) to evaluate patch test reactions to p-Phenylenediamine and their relevance, when tested alone or simultaneously with para-aminoazobenzene. With and without simultaneous testing with para-aminoazobenzene, 4.3% and 3.1% of the patients reacted to p-Phenylenediamine, respectively. The reactions were estimated as relevant in 21.1% and 39.7% of the patients, respectively, with and without simultaneous testing with para-aminoazobenzene.

Dickel et al. (2001) conducted a study to determine differences in sensitization rates between 2 racial groups undergoing patch testing over a period of 4 years. A retrospective computer review of the standard screening tray results of 991 patients (877 whites [88.5%]; 114 blacks [11.5%] average age: 45.9 years) was completed. The patch test methodology involved the application of Finn chambers to the back for 48 hours; reactions were scored at day 3 and between days 4 and 8.

Nickel sulfate and thiomersal and nickel sulfate and p-Phenylenediamine were the 2 most common sensitizers among whites and blacks, respectively. There was a statistically significant difference (P = 0.00599) in the sensitization rate for p-Phenylenediamine in black patients (10.6%) when compared to white patients (4.5%). There were also statistically significant differences in sensitization rates for p-Phenylenediamine (21.2%; P = 0.00005) and imidazolidinyl urea in petrolatum (9.1%; P = 0.04103) in black men when compared to white men (p-Phenylenediamine [4.25%] and imidazolidinyl urea [2.6% in petrolatum]).

The authors stated that the differences in sensitization rates, especially for p-Phenylenediamine, may reflect variations in allergen exposure among racial groups or interindividual variations in the N-acetylation (N-acetyltransferase 1 [NAT1] and 2 [NAT2]) capacities of human skin for p-Phenylenediamine (Dickel et al. 2001).

DeLeo et al. (2002) examined the differences in patch test results between black and white individuals patch tested by members of the NACDG) from July 1, 1992 to June 30, 1998. Patches (Finn chambers on Scanpor tape) were applied to the upper back and remained in place for 48 hours. Reactions were scored at 48 to 72 hours initially, and, again, between 72 and 168 hours.

A total of 9624 patients was patch tested with various allergens during 3 2-year periods, and the sensitization rates

for 1% p-Phenylenediamine in petrolatum were as follows: 1992-1994 (black: 7.8%; white: 5.8%); 1994-1996 (black: 13.5%; white: 5.8%, P <0.05 - significant difference); 1996-1998 (black: 10.3%; white: 5.3%, P <0.05). Differences between white and black patients in their responses to specific allergens were noted.

The authors stated that these differences, although possibly related to genetic factors based on race, are more likely related to differences in allergen exposure that are based on ethnicity (DeLeo et al. 2002)

Li and Wang (2002) studied contact hypersensitivity in hand dermatitis by patch testing 105 consecutive adult hand dermatitis patients (29 males, 76 females; average age: 41.8 ± 15.5 years) and 361 cases of suspected non-hand allergic contact dermatitis (101 males, 260 females; average age: 41.5 ± 22.4 years). The test substances (allergens) were applied to the upper back using Finn chambers; results were recorded at 2 and 3 days. Reactions in the hand dermatitis group were lower than in the non-hand dermatitis group (46.7% versus 63.2%, $p < 0.01$). p-Phenylenediamine was among the most common allergens, at 14.3%. The incidence of reactions to p-Phenylenediamine in the non-hand dermatitis group was 14.1%.

Uter et al. (2002) patch tested 638 patients between January of 1995 and December of 1999. Positive reactions were based on scores at 72 hours. More frequently, positive reactions to the following chemicals were observed: p-aminoazobenzene (16.2%), p-Phenylenediamine (14.1%), p-toluylenediamine (10.0%), 4,4'-diaminodiphenylmethane (8.5%), Disperse Orange 3 (8.4%), and p-aminophenol (3.1%).

In a multicenter study, Krasteva et al. (2002) investigated the validity of the open test ("skin sensitivity test" or "dab test") that is recommended 48 hours before hair dye application as a practical method for detecting allergic reactions to p-Phenylenediamine-containing hair dyes. The study groups consisted of 30 contact dermatitis patients (28 females, 2 males; mean age: 40 years) with positive patch test reactions to p-Phenylenediamine and 30 sex- and age-matched p-Phenylenediamine-negative subjects with no history of adverse reactions to hair coloring products. In an open patch test, a hair coloring product containing 1.8% p-Phenylenediamine was applied to the retroauricular area of each subject and remained for 48 hours (no washing of site). Reactions were scored on day 0 (at 1 hour post-application) and on days 2 and 4.

Allergic reactions were observed in all p-Phenylenediamine positive patients (maximal intensity on day 2). Allergic reactions were not observed in any of the p-Phenylenediamine negative subjects (Krasteva et al. 2002).

Chey et al. (2004) conducted a 2-phase study to examine patients with lichen simplex chronicus (LSC) or other types of neurodermatitis and determine any association with hair dyeing. In a 14-month prospective study, patch testing was performed on 27 patients (18 females, 9 males; mean age of 48.4 years) who dyed their hair regularly and had clinical manifestations of LSC. Finn chambers (on Scanpor tape) were applied to the back, and reactions were scored at day 2 and day 4 (or day 3) according to ICRDG recommendations. Of the 27 patients, 11 patients (40.7%) had positive reactions (2 ++ and 9 + reactions) to p-Phenyelendiamine (test concentration not stated). The 11 patients had been using hair dye for more than 5 years at a frequency of 1 to 3 months.

In a retrospective study, 560 patients (mean age not stated) were examined from March of 1997 to April of 2001. This group comprised patients suspected of having allergic contact dermatitis and further selected cases with concurrent LSC and/or other neurodermatitis. Of the 560 patients patch-tested (same procedure), 63 (11. 3%) had weakly positive reactions to p-Phenylenediaminme (test concentration not stated). Fourteen (22.2%) of the 63 patients had clinical findings of LSC and were also using hair dyes (use frequency = 1 to 3 months) (Chey et al. 2004).

Dawe et al. (2004) reviewed the results of all patients patch tested between 1990 and 2000 at St. John's Institute of Dermatology in the United Kingdom. A total of 13,300 patients with suspected contact dermatitis were patch tested with the European standard series. The records of all patients with a positive patch test reaction to 1% p-Phenylenediamine-free base in petrolatum (from January of 1990 to December of 1999) were obtained. The total number of positive reactions to p-Phenylenediamine (+ to +++) over the 10-year period was 449 (3.4% of the 13300 patients).

Lewis et al. (2004) searched patch-test databases at 2 dermatology centers in the United Kingdom (years 1993 to 2003; total population = 677,500) for all patients who were ≤16 years of age and had a positive patch test to the European standard series. The results yielded 191 consecutively patch tested children, and the most common allergens in these patients were as follows: nickel (13% of the 191 patients), followed by fragrance (9%), thiuram (9%), cobalt (8%), p-Phenylenediaimine (6%), tixocortol pivalate (5%), and myroxylon pereirae resin (5%).

Pratt et al. (2004) reported the results of patch testing from January 1, 2001 to December 31, 2002, by the North American Contact Dermatitis Group. The patients (2 to 97 years old) were patch-tested with a screening series of 65 allergens. Patch testing was done with a standardized technique using Finn chambers on Scanpor tape. The patches remained in place for 48 hours, and test sites were evaluated initially at 48 to 72 hours, and, again, between 72 and 168 hours after placement. A positive reaction was interpreted as +, ++, or +++, manifested by erythematous

papules, vesicles and/or a spreading reaction, sometimes with crusting and ulceration. The clinical relevance of the positive patch test reactions was determined by the patient's history and clinical skin examination findings.

Of the 4,903 patients patch tested with 1% p-Phenylenediamine in petrolatum, 4.8% had positive reactions, 0.2% had irritant reactions, and 0.5% had reactions that were classified as unknown (Pratt et al. 2004).

Wetter et al. (2005) compared the results of patch testing with the standard series at the Mayo Clinic (from July 1, 1998 to December 31, 2000) with those of the NACDG for the same period. The Mayo Clinic patient group consisted of 1320 patients (mean age = 54.7 years) and the NACDG study consisted of 5831 patients. The methodology for patch application (Finn chambers on Scanpor tape) and reading timesused by the Mayo Clinic were identical to those used by the NACDG. Patches were applied to the upper back and remained for 48 hours. Reactions were scored at 48 to 72 hours initially, and, again, at 96 to 168 hours. A positive allergic patch test result was defined as a weak, strong, or extreme reaction, or as a macular erythema reaction if the result was relevant. The positive reaction rate for p-Phenylenediamine (1% in petrolatum) was 4.9% for both the 1,320 patients in the Mayo Clinic study group and the 5,831 patients in the NACDG study group.

Krasteva et al. (2005) investigated the sensitivity and specificity of the skin allergy test (a consumer test) to detect and prevent contact allergy to oxidative hair coloring products that contained a range of concentrations of p-Phenylenediamine and corresponded to different shades (light, medium, and dark). The skin allergy test consisted of the open application of the colorant base prior to mixing with the developer, which is recommended at 48 hours prior to hair coloring. Thirty-four subjects (age range: 23 to 68 years) with allergic reactions to p-Phenylenediamine and 49 non-allergic subjects were involved in the study. The experimental group consisted of subjects with positive patch test reactions (+ to +++) to p-Phenylenediamine during routine investigations during the last 5 years. Test coloring products that contained increasing concentrations of p-Phenylenediamine (0.1, 0.5, 1.0, or 1.5%) were applied to both groups of subjects.

Open patch test results indicated allergic reactions to the test products containing p-Phenylenediamine (at day 2 post-application) in each of the 34 subjects with a history of positive patch test reactions to p-Phenylenediamine. All 34 subjects developed definite type IV allergic reactions (erythema and papules or homogeneous infiltration) to the test products. Allergic reactions were not observed in the 49 control subjects. Of the 34 subjects, 27 had a definite allergic reaction (allergic contact dermatitis) to product A (light shade prototype, 0.1% p-Phenylenediamine), and the reactions were evaluated as follows: weakly positive (2 of 27 subjects), moderately positive (12 of 27), or strongly positive (13 of 27). Three subjects had a positive reaction to product B (contained 0.5% p-Phenylenediamine) and 3 subjects also had a positive reaction to product C (contained 1% p-Phenylenediamine). One subject had a positive reaction to product D (dark shade prototype, 1.5% p-Phenylenediamine).

The authors explained that, because a more severe reaction may be expected following the application of a product containing p-Phenylenediamine at a concentration greater than the first eliciting concentration, they refrained from applying higher concentrations whenever a subject developed a clear-cut contact allergic reaction to the test products. Thus, if one considers that 27 subjects completed the study after reacting to product A (contained 0.1% p-Phenylenediamine), only seven subjects remained to be tested with products containing higher concentrations of p-Phenylenediamine. With this in mind, the authors reported that the cumulative response rates for subjects with reactions to the products were as follows: 0.1% p-Phenylenediamine (27 of 34 subjects), 0.5% p-Phenylenediamine (30 of 34), 1.0% p-Phenylenediamine (33 of 34), or 1.5% p-Phenylenediamine (34 of 34 subjects), corresponding to 79, 88, 97, or 100% of the study subjects, respectively (Krasteva et al. 2005).

Wang et al. (2005) analyzed cosmetic allergic contact dermatitis (CACD) in eczema patients in China and examined the frequency of patch test reactions to common cosmetic-related allergens (CRA). Three-hundred seventy-eight consecutive eczema patients (120 males, 258 females; average age = 40.5 ± 16.4 years) in China were patch-tested with a modified European standard series of allergens during a 2-year period.

Of the 378 patients, 73 (19.3%) were suspected of having CACD and 37 patients (9.8%) were classified as confirmed. The frequencies of the positive patch test reactions in patients suspected of having CACD and in those with confirmed CACD to at least 1 CRA were 64.4% and 89.2%, respectively.

The values associated with p-Phenylenediamine patch tests were 5.5% and 8.1%, respectively; the values associated with fragrance mix patch tests were 27.4% and 32.4%, respectively. The results of this study suggested that CACD is very common in patch-tested eczema patients in China. p-Phenylenediamine and fragrance mix were the leading allergens that were identified (Wang et al. 2005).

A retrospective analysis by Oppel and Schnuch (2006) was based on 2004 patch test results that were collected by the Information Network of Departments of Dermatology in Germany. Data on 9,948 patients (6,175 females; 3,773 males) were referred for evaluation of suspected contact allergy to determine the frequency of sensitization to the 10 most common contact allergens. The top 10 allergens were

as follows: nickel sulfate (frequency of sensitization, standardized for sex and age: 17.2%), fragrance mix (sensitization frequency: 7.2%), balsam of Peru (6.7%), cobalt chloride (6.5%), potassium dichromate (5.3%), colophony (4.6%), lanolin alcohol (4.3%), p-Phenylenediamine (4.2%), ammoniated mercury (3.5%), and methyldibromoglutaronitrile/phenoxyethanol (3.4%).

White et al. (2006) studied the role of Bandrowski's base in p-Phenylenediamine-induced allergy. The purity of the test substance was 99.9%. The main impurity was Bandrowski's base at 0.1%, and traces of an organic impurity, tentatively identified as 4,4'-azodianiline, were also reported. A 7-month stability analysis indicated an increase in the concentration of Bandrowski's base from 0.1% to 0.5%. In patch tests, test substances were applied in Finn chambers.

Of the 159 patients attending a dermatology clinic in Bangkok, 8 were patch test positive to p-Phenylenediamine. Only 1 patient had a positive reaction to 1% Bandrowski's base, and this reaction was observed in the patient who had a strong positive reaction (+++) to p-Phenylenediamine.

Additional tests involved 6 subjects who had previously attended the dermatology clinic in London and 29 patients in Bangkok (patients from both clinics patch tested positive to p-Phenylenediamine). Reactions in the 6 subjects (London) who originally had + reactions to p-Phenylenediamine were as follows: 0.01% p-Phenylenediamine (1 patient), 0.1% p-Phenylenediamine (3 patients), 1% p-Phenylenediamine (5 patients), and no reactions to any concentration of p-Phenylenediamine (1 patient). All 6 were negative to Bandrowski's base.

Twenty-six of the 29 subjects (Bangkok) had 1+ reactions to p-Phenylenediamine. Only 5 of the 29 subjects reacted to Bandrowski's base. Of the 642 normal volunteers (in Bangkok) patch-tested, 14 had positive reactions to p-Phenylenediamine. Five of the 14 also had positive reactions to 1% Bandrowski's base (White et al. 2006).

Uter et al. (2006) conducted a study to determine whether aniline should be regarded as a potential cause of contact allergy. A retrospective analysis of clinical data collected in a contact allergy surveillance network (Information Network of Departments of Dermatology) between January of 1992 and June of 2004 was performed. The patch test procedure was said to follow current international guidelines, further amended by the German Contact Dermatitis Research Group. During this period, 25 (mean age = 57) of 119 patients patch tested with aniline (1% in water or petrolatum) had positive (allergic) reactions. The median age of the patients who tested negative was 43. Of the 25 patients, 24 were diagnosed with contact allergy to p-Phenylenediamine (1% in petrolatum).

Jowsey et al. (2006) conducted a study to assess the elicitation response characteristics observed in p-Phenylenediamine-allergic volunteers patch tested with complete hair dyes. Three groups of 15 volunteers (≥ 18 years) with + (group 1), ++ (group 2), or +++ (group 3) reactions to p-Phenylenediamine at 2 days after patch removal participated in the study. p-Phenylenediamine (1% in petrolatum) was applied to the arm for 48 hours in Finn chambers. Reactions were scored at day 2 post-application according to the ICDRG scale. Subsequently, each group was patch tested with 2 complete hair dyes (A and B, both permanent black hair dyes) for 30 minutes, 1 hour, and 24 hours. Reactions were scored at days 1, 2, and 3 after patch removal.

The authors stated that exposure to either complete hair dye A or B for 30 minutes or 1 hour was insufficient to yield a positive patch test reaction in all of the p-Phenylenediamine-allergic patients in group 1 or group 2. Additionally, the application of either hair dye for 24 hours was sufficient to yield positive reactions in all of the volunteers in group 3 and group 4. Following 24-hour exposure to complete permanent hair dyes, the frequency of positive patch test reactions was comparable to that observed after 48-hour exposure to 1% p-Phenylenediamine in petrolatum in volunteers whose degree of sensitization was such that they typically present with ++ or +++ reactions diagnostically (Jowsey et al. 2006).

Søsted et al. (2006a) evaluated the dose-effect elicitation of allergic contact dermatitis in p-Phenylenediamine allergic patients using 48-hour patch tests. The study involved 15 patients (14 female, 1 male; 24 to 64 years old) who had a positive patch test reaction to p-Phenylenediamine over the period encompassing 1999 to 2003. All were allergic to p-Phenylenediamine. The study was conducted at the Department of Dermatology at Gentofte Hospital in Denmark from January to July of 2005. The test substance (1% p-Phenylenediamine in white petrolatum) and serial dilution of the test substance to concentrations ranging from 1 ppm to 10,000 ppm were evaluated. White petrolatum served as the negative control. Finn chambers on Scanpor tape were applied for 48 hours, and reactions were scored on days 3 and 7 according to the ICDRG's grading scale: 0 (negative) to 4 (strongly positive). Test sites included the back, outer aspects of the arms, and retroauricular area.

One female subject did not have a response to 1% p-Phenylenediamine in white petrolatum, considering that a ++ reaction to the test substance was observed 6 years ago. The remaining patients had at least a +? (weakly positive) reaction to 1% p-Phenylenediamine in white petrolatum. Seven of the 15 patients also had other contact allergies. The threshold value for 10% of the patients tested (ED_{10}), based on + reactions or greater on the back, was 38 ppm. For each of the 3 regions (back, ears, and arms), dose-response curves were drawn. Statistically significant differences in the sensitivity of the 3 anatomical regions were not found. The authors stated that the upper back is a

suitable region for patch testing patients with hair dye dermatitis (Søsted et al., 2006a).

Clayton et al. (2006) conducted a retrospective study to determine whether the site of primary dermatitis in children could predict a diagnosis of allergic contact dermatitis. Between the years 1995 and 2004, 500 children were patch tested. The age groups ranged from 0 to < 2 years to ≥ 15 years. The allergens were applied, under Finn chambers secured with Scanpor tape, to healthy skin on the back. The patches remained in place for 48 hours and reactions were scored at 48 and 96 hours according to ICDRG criteria.

Of the children tested, 133 (27%) had 1 or more positive patch test reactions. The most frequent finding was type IV allergy to nickel (33% of the children). Reactions to fragrance mix (18%), cobalt (11%), mercapto chemicals, *Myroxylon pereirae*, and p-Phenylenediamine (each 8%) were also reported. No statistical significance was found regarding the relationship between the site of primary dermatosis and a positive patch test result (Clayton et al. 2006).

Hillen et al. (2006) assessed the frequency of late reactions to p-Phenylenediamine and epoxy resins in 1,748 patients (data on 1428 patients were evaluable). Diglycidyl ether of bisphenol A (1% in petrolatum) and p-Phenylenediamine (1% in petrolatum) were among the chemicals that were tested. Both substances were applied to the medial side of the upper arm. Patch test occlusion time was 24 hours (588 patients) or 48 hours (1,160 patients). For 24-hour patch tests, reactions were scored on days 1-3; for 48-hour patch tests, reactions were scored on days 2-3. Late readings were made on days 7, 14, and 21 after patch application. Reactions were scored according to ICDRG criteria.

Of the 1,428 patients for which data were evaluable, patch tests for 25 patients were not positive before day 7. These included 21 reactions to p-Phenylenediamine (1.5% of the 1,428 patients) and 4 reactions to diglycidyl ether of bisphenol A (1% in petrolatum). For 5 of the 7 patients, repeated patch tests with p-Phenylenediamine revealed patch-test sensitization as the cause of the late reaction. With the exception of 1 reaction, all late reactions were observed in patients in whom patches were applied for 48 hours. The authors concluded that p-Phenylenediamine (1% in petrolatum) elicited late reactions in 1.5% of routine patch tests, and that the majority of the late reactions was caused by patch-test sensitization (Hillen et al., 2006).

Aalto-Korte et al. (2007) reported late patch test reactions to p-Phenylenediamine. A late reaction was defined as a reaction that was noticed (by the patient or someone else) at least 10 days after patch application, and which was negative at the last reading on days 4 through 6. Patch tests were performed according to ICDRG guidelines using the Finn chamber method. Between January of 2002 and February of 2006, 826 patients were patch tested with p-Phenylenediamine (1% in petrolatum). Of the 826 patients, 26 (3.1%) had positive reactions to p-Phenylenediamine. Late reactions were observed in 6 patients (0.75% of 826 patients).

Hillen et al. (2007) patch-tested 1,222 patients (ages not stated), with suspected allergic contact dermatitis of the scalp, with 1% p-Phenylenediamine in petrolatum. Patch tests were read according to ICDRG criteria. Of the 1,222 patients, 144 (11.8%) had positive reactions to the test substance.

In a study by Patel et al. (2007), 6177 patients with suspect contact dermatitis were patch tested from January of 1999 to December of 2004 using standard ICDRG criteria. Reactions were scored at 2/3 days and 4/5 days. The patch test frequency (%) to p-Phenylenediamine was ascertained for each year. A statistical analysis was performed using a Chi-Squared and Cochrane-Armitage trend test. Results indicated that the proportion of patients that tested positive to p-Phenylenediamine ranged from 3.8% in 1989 to 7.1% in 2004, and, thus, was not constant ($P < 0.001$). A Cochran-Armitage linear trend test was applied to these data, and the P-value of <0.001 that was determined means that the lack of homogeneity in proportions could be attributed to a linear increase over time.

White et al. (2007) compared elicitation reactions following single and intermittent exposure to p-Phenylenediamine. Both single-dose and repeated exposure involved the same 23 patients (14 females, 9 males; 25 to 68 years old) who were classified as allergic to p-Phenylenediamine. In the single dose experiment, p-Phenylenediamine was applied to different sites on the upper back at the following concentrations on each successive day: 0.3% in petrolatum, 0.3% aqueous, 0.03% in petrolatum, and 0.03% aqueous. The test concentrations were applied for 5 min on day 1, for 10 min on day 2 and onward up to a 40 min application as a single exposure. In the repeated exposure experiment, the same concentrations of p-Phenylenediamine base were used; however, 5-min applications were made on 8 successive days (same site).

Reactions to either 0.03% aqueous p-Phenylenediamine or 0.03% in petrolatum were not observed after single or repeated applications. Eight reactions to 0.3% aqueous p-Phenylenediamine were observed at the cumulative exposure site. For 7 of the reactions, there was an exact correlation between the reaction to the cumulative time needed for repeat exposures to elicit a reaction and the time needed for a reaction to the single exposure. There was also a close correlation between the grade of the original reaction and the reaction frequency to 0.3% p-Phenylenediamine in petrolatum.

These results, together with the results of the skin binding study (rats) by these authors discussed earlier, demonstrate that, over the time period tested, the allergenic component

of p-Phenylenediamine accumulates in the skin. Therefore, the authors concluded that a smaller cumulative time exposure is equivalent to a larger single-time exposure in this study (White et al. 2007).

p-Phenylenediamine HCl

Picardo et al. (1990) conducted patch tests involving freshly prepared solutions of p-Phenylenediamine HCl and 3 selected aromatic compounds that are structurally similar to p-Phenylenediamine (p-aminophenol, o-aminophenol, hydroquinone). Eighty patients (ages not stated) who were positive to at least one hapten of the para group (p-Phenylenediamine, diaminodiphenylmethane, benzocaine, p-Phenylenediamine mix) participated in the study. The number of positive reactions correlated with the rate of decomposition of the substances, as evaluated by high-pressure liquid chromatography. p-Phenylenediamine HCl, which was almost decomposed after 24 hours, gave the highest number of positive reactions, followed by o-aminophenol and by p-aminophenol, while hydroquinone (oxidized to the extent of 35%) did not give any reactions. Patch test results for 0.5% p-Phenylenediamine HCl indicated 27 positive reactions.

Zhao and Fan (1991) patch tested 107 patients (89 females,18 males; ages 2 to 65 years) with facial contact dermatitis. Patches (Finn chambers) remained in place for 48 hours (Finn chambers; 48-hour application) and reactions were scored at 48, 72, and 96 hours. p-Phenylenediamine HCl was the most frequent contact allergen (17 patients with positive reactions).

Guerra et al. (1992a) patch tested 261 hairdresser clients (5 males, 256 females; mean age: 43.3 years) from 1985 to June of 1990. Of the 261, 176 had dermatitis. Reactions were scored at 2 and 3 days. Among hair dye allergens, p-Phenylenediamine was the most frequent sensitizer (7.3%; 19 patients with positive reactions to 0.5% p-Phenylenediamine HCl in petrolatum).

Lee and Lam (1996) patch-tested 490 eczematous patients with European standard allergens and suspected causative substances that were brought in by the patients. Of the 490 patients, 437 completed the tests. The 437 patients were classified as follows: 256 (with contact dermatitis), 109 (with endogenous eczema), and 72 (with unclassified eczema). Patch tests were conducted using Finn chambers secured with surgical tape. Reactions were scored at days 2 and 4. p-Phenylenediamine HCl was tested at a concentration of 0.5%. Of the 256 contact dermatitis patients patch-tested, 1 had an allergic reaction to 0.5% p-Phenylenediamine HCl. No reactions to 0.5% p-Phenylenediamine HCl occurred in the remaining 2 patient groups.

p-Phenylenediamine and p-Phenylenediamine HCl

Sertoli et al. (1999) conducted a multicenter survey involving 42,839 contact dermatitis patients (ages not stated). The study was initiated in 1984. p-Phenylenediamine HCl (0.5%) was tested from 1984 to 1988, and p-Phenylenediamine base (1%) was tested from 1989 to 1993. The patch tests were applied to the back and remained in place for 48 hours. Reactions were scored initially at 20 to 60 minutes after patch removal and 24 and 48 hours after the first reading. Of 42,839 patients patch tested (1984 to 1993), there were 1481 positive reactions.

Cross-sensitization

As reported by Baer et al. (1973), Baer (1976), Fisher (1976), Morikawa et al. (1976), Herve-Bazin et al. (1977), Rudzki (1977), Price and Shupack (1978), Epstein and Taylor (1979), and Klein and Rodman (1981), allergic sensitivity to p-Phenylenediamine has been associated with cross sensitization to:

- azo and aniline dyes;
- local anesthetics, procaine and benzocaine;
- p-aminobenzoic acid, its esters, and sunscreens containing them;
- IPPD, used in rubber tires;
- CPPD;
- p-aminosalicylic acid;
- hydrodiuril;
- carbutamide;
- pyrogallol;
- sulfonamides;
- hydroquinone;
- hydrochlorothiazide;
- p-hydroxybenzoic acid esters;
- benzidine;
- phenylhydrazine; and
- p-toluenediamine.

Storrs et al. (1979) observed cross reactivity between p-Phenylenediamine and p-Phenylenediamine HCl. Eleven men and 11 women who were sensitive to 1% p-Phenylenediamine in petrolatum were patch tested with 1% p-Phenylenediamine HCl in petrolatum. Only 3 of the men and 6 of the women were sensitive to p-Phenylenediamine HCl. No subjects who were negative to p-Phenylenediamine were positive to p-Phenylenediamine HCl.

Turchin et al. (2006) evaluated the rate of cross-reactivity between parabens, p-Phenylenediamine, and benzocaine in a population of patients patch-tested in a hospital-based dermatitis clinic. A retrospective analysis of 4,368 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 uper back and remained for 2 days. Reactions were scored after 48 and 96 hours according to ICDRG guidelines.

The positive reactions in the group of 4,368 patients were reported as follows: 253 (5.7%) patch-test positive to p-

Phenylenediamine, 37 (0.8%) patch-test positive to benzocaine, and 34 (0.7%) patch-test positive 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%) (Turchin et al. 2006).

Genetic Susceptibility to Sensitization

Several studies reported in the section on provocative testing suggested a possible genetic component to p-Phenylenediamine sensitization. The studies below further address that question.

p-Phenylenediamine

Brans et al. (2005) performed a case-control analysis to compare the distribution of 2 polymorphisms in genes encoding for the manganese-containing superoxide dismutase 2 in mitochondria (MnSOD) in patients with a confirmed sensitization to p-Phenylenediamine to determine whether these polymorphisms have an influence on the individual's susceptibility. The authors noted that MnSOD is one of the primary enzymes that directly scavenge potentially harmful oxidizing species, and that a valine (Val) to alanine (Ala) substitution at amino acid -9 (located in MnSOD gene) has been associated with various disease risks.

The aim of this study was to investigate possible associations of the MnSOD 47 T>C (thymine to cytosine base pair transition) genotype in exon 2 (Ala-9Val) and the 339 T>C genotype in exon 3 (ILe58Thr) with contact sensitization to p-Phenylenediamine in humans. The cases that were recruited for the study included 157 females (median age = 45 years, range: 11 to 96 years). Each of the 157 female subjects had a positive patch test reaction to p-Phenylenediamine (reactions ranged from 1+ to 3+). The controls (n = 201) that were recruited did not have a history of sensitization to p-Phenylenediamine, and were age- and gender-matched to the cases.

Study results indicated no heterozygous (CT) or homozygous (TT) carriers for the Ile58Thr polymorphism. Furthermore, frequency values for the C allele of the Ala-9Val polymorphism were 51% (79 of 157 cases) and 49% (107 of 201 controls). Values for the incidence of homozygous CC carriers (Ala/Ala) were 27% (43 of 157 cases), and 23% (46 of 201 controls; odds ratio (OR) = 1.3; 95% confidence interval (CI) = 0.8 to 2.1). An increased risk for sensitization among homozygous CC carriers (Ala/Ala) was reported only for the group of older women (> 45 years, 25% versus 18%; OR = 1.5; 95% CI = 0.7 to 2.34). The authors stated that these data suggest that the C (Ala) allele of MnSOD modifies the contact dermatitis risk in older females, but is not an independent susceptibility factor for contact sensitization to p-Phenylenediamine (Brans et al., 2005).

Nacak et al. (2006) conducted a study to evaluate whether genetic polymorphism of NAT2 plays a role in the individual susceptibility to p-Phenylenediamine sensitivity. Seventy contact dermatitis patients (42 females, 28 males; median age = 38 years) participated in the study. The control group consisted of 100 age- and sex-matched controls (40 males, 60 females; median age = 34 years). These subjects (controls) did not have contact dermatitis or signs of atopic disease, allergic or lung disorders, and diseases that may have an association with the acetylation polymorphism, such as diabetes mellitus and cancer. The frequencies of 7 NAT2 point mutations were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.

The study results indicated that the genotypes coding rapid acetylation were found in 52.9% and 37.0% of the patients with contact dermatitis and control subjects, respectively. Additionally, the frequency of the NAT2*4 allele and NAT2*4/*4 genotype, coding for rapid acetylation, were also significantly higher in the contact dermatitis patients than in the control subjects (P = 0.003). The results of this study suggest an association between rapid acetylation polymorphism and susceptibility to p-Phenylenediamine sensitization (Nacak et al. 2006).

Photosensitization

p-Phenylenediamine

Horio (1976) performed patch and photopatch tests with 2% p-Phenylenediamine in petrolatum on the back of a 52-year-old man. The photopatch site was irradiated for 15 minutes at a distance of 15 cm, 24 hours after application. The sites were scored at 24 and 48 hours after irradiation. The light source was a black light (Toshiba FLZOBLB) emitting wavelengths from 300 to 420 nm and consisting mainly of long-wave UV peaking at 360 nm. The papulovesicular reactions observed at the patch and photopatch sites were approximately equal.

Thune (1984) conducted a photosensitization study involving 23 patients (15 females, 8 males) with eczema, redness, stinging, or burning of the face following sun exposure. Nine of the patients had polymorphic light eruption and 3 had persistent light reactivity. An additional patient had a photosensitivity that was of unknown origin and chronic contact dermatitis. Patch tests were performed and reactions were scored at 48 and 72 hours. For photopatch testing, a 150W xenon solar simulator, equipped

with Schott filter WG345 to eliminate UVB, was used (UVA irradiance = 13 mW/cm^2). Photocontact allergy to p-Phenylenediamine was observed in one patient and plain contact allergy was observed in a second patient.

Occupational Exposure

p-Phenylenediamine

A study was conducted with 20 subjects who had suffered allergic contact dermatitis from p-Phenylenediamine and had strongly positive patch tests to p-Phenylenediamine. The subjects were patch tested with hair containing p-Phenylenediamine dyed 24 hours previously. The patches were repeated 3 weeks later. There were no reactions at the end of 48 hours or 3 weeks (Reiss and Fisher 1974; Fisher 1975).

Mainka (1983) reported allergic reactions, caused mainly by chromium, cobalt, and p-Phenylenediamine, in workers at a steel plant. Of the 53 workers (ages = 25 to 58 years) evaluated, 7 had positive reactions to p-Phenylenediamine.

Goh et al. (1984) reported negative patch test reactions to p-Phenylenediamine in 4 patients (ages not stated) who worked at different photographic developing companies.

Matsunaga et al. (1988) patch tested 13 beauticians (mean age = 19) with hand dermatitis between 1982 and 1986. A personal or family history of atopy was common in these patients. Patch testing involved the use of Finn chambers that remained in place for 48 hours. Twelve of 13 subjects had definite positive reactions to p-Phenylenediamine. With the exception of 1 + reaction, all of the reactions were classified as ++.

Matsunaga et al. (1989) patch tested 35 hairdressers and barbers with hand dermatitis in an effort to prevent this occupational skin hazard. Patch testing (open test) was performed during a 23-month period from January of 1987 to November of 1988. Thirty-three of the 35 cases were identified as allergic contact dermatitis. Of these 33 patients, 28 (85%) had a positive patch test reaction to 1% p-Phenylenediamine in petrolatum.

Guerra et al. (1992b) conducted a multicenter study to evaluate the frequency and source of contact sensitization in a group of 302 hairdressers (43 males, 259 females; mean age: 24.6 years) with dermatitis. In patch tests (Finn chambers), reactions were read at 2 and 3 days. p-Phenylenediamine caused 73 positive reactions (24.2%).

Sutthipisal et al. (1993) investigated the potential for atopics to be sensitized to contact allergens. The study group consisted of 143 hairdressers (18 men, 125 women) with hand eczema. Patch tests (1% p-Phenylenediamine, Finn chambers) were applied for 48 hours and reactions were scored at 2 and 4 days . The following positive reactions to 1% p-Phenylenediamine were reported: 11 of 45 eczematous atopics (24% incidence) 11 of 32 mucous membrane atopics (34% incidence) and 22 of 66 non-atopics (33% incidence).

Frosch et al. (1993) patch tested 809 hairdressers in a multicenter study. The leading allergens were glyceryl monothioglycolate (sensitization rate = 19%) and p-Phenylenediamine (sensitization rate = 15%).

Katsarou et al. (1995) patch tested 106 hairdressers (102 females, 4 males; mean age = 23.7 years) with contact dermatitis. Thirty-two positive reactions to 0.5% p-Phenylenediamine (in petrolatum) were reported.

Rademaker (1998) patch tested 46 farmers (21 males, 25 females; mean age: 59 years) over a 4-year period to determine whether their dermatitis was secondary to an occupational allergen. Four of the farmers (all with hand dermatitis) had positive patch test reactions to rubber compounds. Of these, 2 had a positive reaction to p-Phenylenediamine.

In a study by Leino et al. (1998a), 71 female hairdressers with contact dermatitis were diagnosed during 1974 to 1993. Positive reactions to p-Phenylenediamine were observed in 5 of 65 (7.7%) patients who were tested.

Leino et al. (1998b) studied the occurrence and causes of hairdressers' occupational skin and respiratory diseases. The study group consisted of 355 hairdressers (15 to 54 years old). Of the 189 subjects who reported work-related skin and respiratory symptoms , 130 underwent a physical examination, lung function tests, prick and patch testing, and nasal and lung provocation tests. p-Phenylenediamine, natural rubber latex, and skin irritation were listed as some of the causes of hand dermatitis. Patch test (48-hour application) results for 54 hairdressers revealed 2 positive reactions to p-Phenylenediamine. Reactions were scored at time of removal and, also, 24 and 48 to 120 hours later.

Lodi et al. (2000) patch tested 1,565 out-patients (1,056 females, 509 males) with dermatitis who had various occupations (e.g., 41 hairdressers tested). The dermatitis was suspected of being job-related. The allergen with the highest frequency of positive reactions in the 1,565 patients was p-Phenylenediamine (25.3% = frequency of positive reactions). Of the 41 hairdressers (34 females, 7 males) patch tested, 15 had positive patch test reactions to p-Phenylenediamine (36.5% = frequency of positive reactions).

Dickel et al. (2002) reported standard patch test results for 4,112 employed persons with an initial report of occupational skin disease over a 10-year period. p-Phenylenediamine induced sensitization in 10.7% of the patients (440 patients).

Fautz et al. 2002 investigated the cross reaction pattern of new generation hair dyes (FD & C and D & C dyes) that are used in hair dye formulations among hairdressers (n = 40). Each hairdresser had a known relevant reaction to p-Phenylenediamine and/or 2,5-diaminotoluene sulfate and/or 2-Nitro-p-Phenylenediamine. The 40 hairdressers were patch tested with the individual dyes (total of 11, highest non-irritant concentration), the hair dye formulations (total of 8, highest non-irritant concentration), and with p-Phenylenediamine (1.0% in petrolatum), 2,5-diaminotoluene sulfate (1.0 % in petrolatum), and 2-Nitro-p-Phenylenediamine (1.0% in petrolatum).

In the 40 hairdressers tested, there were no positive reactions to any of the individual dyes at day 2 or day 3. Thirty-eight of the hairdressers did not have positive reactions to any of the hair dye formulations at day 2 or day 3. The results of this study suggest that, for hairdressers sensitized to p-Phenylenediamine and/or 2,5-diaminotoluene sulfate and/or 2-Nitro-p-Phenylenediamine, the new generation of hair dyes is a safe alternative for use in their salons (Fautz et al. 2002).

Iorizzo et al. (2002) conducted a study to evaluate the frequency, age distribution, and source of contact sensitization in a group of 209 hairdressers (182 females, 27 males; mean age: 14 to 72 years) who visited a dermatology clinic in Bologna Italy. The mean duration of the dermatitis was 1.75 year. Patch tests were conducted using Finn chambers on Scanpor tape, and reactions were scored on days 2 and 3 according to the following scale: + (erythema with infiltration) to +++ (erytherma with innfiltration, papules, and vesicles). Of the 209 hairdressers patch tested, 132 had one or more clinically relevant positive reactions to different allergens. The hapten that most frequently caused positive reactions among the 209 patients was 1% p-Phenylenediamine base in petrolatum (77 patients, 36.8% of patients tested).

Uter et al. (2003) conducted a study to determine whether the pattern of sensitization to cosmetic ingredients was different in hairdressers (884 patients; median age = 24) versus their clients (1,217 patients; median age = 46). The 2 groups included female contact dermatitis patients who consulted with the participating centers of the Information Network of Departments of Dermatology (IVDK). The patients were patch tested between January of 1995 and December of 2002. In order to quantify the risk of specific contact allergy in hairdressers versus clients and to identify possible variations over time (while controlling for age as a potential cofounder), logistic regression analyses were performed for each allergen that was tested. The strength of association was quantified with the odds ratio (OR), which was accompanied by 95% confidence intervals (CIs).

Study results indicated that the proportion of p-Phenylenediamine-allergic hairdressers decreased steadily, but not significantly (OR: 1.30; CI: 1.00 to 69). However, in contrast, a significant increase in p-Phenylenedianine contact allergy was noted in clients ($P < 0.05$, adjusted for age) (Uter et al. 2003).

Li et al. (2003) investigated 14 patients (ages not stated) with occupational allergic contact dermatitis over a period of 2 years (2001-2003). Patch tests were conducted using Finn chambers; reactions were scored at days 2 and 3 according to ICDRG recommendations. Of the 14 patients, positive patch test reactions to p-Phenylenediamine base were reported for 3. Two of the patients were hairdressers and the third patient was a chemist who worked with p-Phenylenediaimine.

In a study by Lind et al. (2005) involving 33 hairdressers (mean age = 37 years), it was concluded that hairdressers' skin is exposed to allergenic compounds during dyeing. Exposure was said to result from dye application, from cutting newly-dyed hair, and from background exposure. The exposure loadings were said to have been at a level where there is a risk of sensitization and/or elicitation of contact allergy (i.e., 22 to 939 nmol per hand for p-Phenylenediamine).

In a study by Valks et al. (2005), 300 hairdressers were patch tested with p-Phenylenediamine and other allergens in the dermatology department of the Instituto Nacional de Medicina y Seguridad in Madrid Spain from 1994 to 2003. Most of the hairdressers were women (mean age = 23.7 years). Patch tests were applied to the upper back and secured with self-adhesive Fixomull for 48 hours. Reactions were scored on days 2 and 4. Results were compared with those from a previous study (Conde-Salazar et al. 1995) involving 379 hairdressers who visited the dermatology department from 1980 to 1993. The present study yielded a significant increase in the frequency of positive patch test reactions (78.3% versus 58.8%) and occupational allergic contact dermatitis (58% versus 48.8%), compared to the previous study.

Positive patch test reactions to 1 or more of the allergens tested were observed in 235 patients (78.3%). Non-occupational allergic contact dermatitis was diagnosed in 16 patients (5.3%). A significant increase in sensitization reactions to most allergens, the following included, was observed: p-Phenylenediamine base (54% versus 45.9%), 4-aminobenzene (40.7% versus 31.9%), ammonium thioglycolate (2.7% versus 2.3%), ammonium persulfate (7.9% versus 14.3%), p-toluenediamine sulfate (6.8% versus 15.3%), p-aminodiphenylamine (2.9% versus 7.7%), o-nitro-4-phenylenediamine (2.1% versus 7.3%), and aminophenols (0% versus 9%). However, a decrease in the number of sensitization reactions to Disperse Orange (17% versus 42.7%) and thioglycolic acid (15.3% versus 3%) was noted. The authors concluded that the high frequency of and increase in sensitization reactions among hairdressers require urgent measures to improve protective measures and their application (Valks et al., 2005).

In a study by Ho et al. (2005), 400 patients (majority in 31 to 60 age group; 109 with history of atopic eczema) developed a positive reaction to p-Phenylenediamine between January of 1995 and April of 2004. More female (68% of patients) than male (32%) patients were involved. These reactions occurred in an average of 47 patients per year. The face and hand were identified as the more common primary sites (in 112 [28%] patients, followed by the hands and what was described as generalized involvement. Of the 400 patients, 36 (9%) were hairdressers. No difference in the severity of the reaction to p-Phenylenediamine was observed when hairdressers were compared with other patients ($p = 0.81$). The majority (53%) of the hairdressers developed a + reaction to p-Phenylenediamine. Ten of the 400 patients had temporary black henna tattoos applied in the past. Three of the 10 developed a ++ reaction and 7 developed a +++ reaction to p-Phenylenediamine.

Hueber-Becker et al. (2007) conducted a study in which 128

healthy hairdressers (125 males, 3 females; mean body weight = 71 ± 12 kg) were exposed to oxidative hair dyes, under controlled conditions, for 6 work days. The hairdressers colored hairdresser's training heads bearing natural human hair with a dark-shade oxidative hair dye containing 2% [^{14}C]-p-Phenylenediamine for 6 hours per work day. Urine and blood samples were collected from all exposed subjects. Liquid scintillation counting was used to determine the level of radioactivity in all biological samples and study materials, tools, and washing liquids, and a [^{14}C]-mass balance was performed daily.

Adverse events were not reported for any of the subjects in the study. Hair + scalp accounted for 53.46 ± 4.06% of the applied radioactivity. The concentration of dye in the plasma was said to have been below the limit of quantification (≤ 10 ng p-Phenylenediamimine$_{eq}$/mL). Total urinary (0 to 48 hours) excretion of [^{14}C] ranged from a total of < 2 to 18 µg p-Phenylenediamine$_{eq}$, and was said to have been similar in subjects that were exposed during the different phases of hair dyeing. The mean mass balance of [^{14}C] for the 6-day study was 102.50 ± 2.20%. The 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 body weight/work day (Hueber-Becker et al. 2007).

p-Phenyelendiamine HCl

Lidén (1988) investigated the occurrence and causes of occupational dermatoses in a population of 114 subjects (ages not stated) exposed to color developing (CD) agents (p-Phenylenediamine derivatives) at a large obsolescent film laboratory. Of the 114, 51 were subjects with previous or present skin disease. The 23 subjects with dermatoses that may have been associated with work in the film laboratory were patch-tested with p-Phenylenediamine HCl (1% in petrolatum). Finn chambers secured with surgical tape were removed after 48 hours and reactions were scored at 72 hours post-application. Reactions were scored again at 2 to 3 weeks later. Two of the 23 subjects had a positive reaction to the test substance. The incidence of positive reactions in dermatitis patients patch-tested with 2 developing agents was as follows: CD-2 (9/23) and CD-3 (4/23).

Sixty-five employees at a modernized film laboratory with a history of chemical exposure and/or dermatitis were patch-tested (same procedure) with p-Phenylenediamine HCl (0.5% or 1% in petrolatum). Only 1 reaction was reported. The incidence of positive reactions in dermatitis patients patch-tested with 2 developing agents was as follows: CD-2 (5/65) and CD-3 (2/65).

Another study was conducted to evaluate the relationship between chemical exposure and lichen planus. The study involved 119 patients (17 to 68 years old) with lichen planus from 2 general dermatology departments. Of the 119 patients, 79 were patch-tested. The test concentration of p-Phenylenediamine HCl in the patch test was not stated. At the 72-hour reading, there were no cases of contact allergy to p-Phenylenediamine HCl. However, a positive reaction was reported at the re-reading after 2 weeks, and at 72 hours during re-testing a year later. Lichen planus was not aggravated by patch testing and typical lichen planus lesions did not develop at the test site. (Lidén, 1988).

De Boer et al. (1989) conducted an epidemiological study involving 286 metalworkers (exposed to metalworking fluids) to determine the prevalence of contact sensitization. Ten metalworking factories in the Netherlands were involved in the study. Thirty-nine of the 286 workers presented with dermatitis of the hands and/or forearms, and an additional 5 recalled a period of hand dermatitis in the past. Forty of the 286 workers were available for patch-testing with a series of common components of metalworking fluids, including p-Phenylenediamine HCl (0.5% in petrolatum). p-Phenylenediamine HCl (0.5% in petrolatum) was applied to the upper back of each of the 40 subjects using a Vander Bend Square chamber, secured with Fixomull tape, for 48 hours. Reactions were scored at 72 hours. Of the 40 workers, 8 had a contact allergy to one or more chemicals. p-Phenylenediamine HCl was not included in the list of chemicals that induced contact allergy.

Case Reports

According to Grant (1974), the use of p-Phenylenediamine-containing hair dyes on the hair has been accompanied by edema of the eyelids and conjunctiva and tearing. Infrequently, there is limitation of eye movements, loss of the corneal epithelium, and cellular infiltration of the stroma. The cornea usually recovers rapidly. More severe reactions occurred after the application of p-Phenylenediamine-containing hair dyes to the eyebrows and eyelashes. Generally, rapid onset of pain and burning of the eyes was accompanied by redness and swelling of the lids and edema and hyperemia of the conjunctiva. In some persons, the corneal epithelium was eroded and accompanied by iritis and iridocyclitis. Vision has occasionally been lost or permanently damaged by severe corneal ulceration. Specific case reports follow.

p-Phenylenediamine

A woman who had dyed her eyebrows and eyelashes with a product containing p-Phenylenediamine developed conjunctivitis within 3 days of application and then developed corneal ulcers in both eyes. Treatment included removal of eyelashes and eyebrows. Two and one-half months following product application, the woman was "feeling better," and 6.5 months following application she was able to see "fairly well" (Moran 1934).

Warin (1976) reported 3 case studies in which men suffered

from eczema on their arms. In 2 studies, the men had positive patch tests to 1% p-Phenylenediamine in petrolatum and to their wives' dyed hair (Cronin 1973; Mitchell 1972). In the third study, the man's eczema flared up whenever his partner had just dyed her hair. Patch tests to 1% p-Phenylenediamine in petrolatum and to the hair dye were positive, and a patch test to his partner's hair dyed 1 week previously was negative. The author suggested that a more recent use of hair dye might have resulted in a positive reaction.

In their review, Marzulli et al. (1978) described a clinical case in which a beautician developed generalized urticaria when working with hair dyes; p-Phenylenediamine caused a wheal- and flare-response on the skin of his back.

Foussereau et al. (1980) observed a dermatitis covering the whole scalp, neck, chest, and both ears in a man who had dyed his hair 1 week previously. Patch tests were positive for 2% p-Phenylenediamine in petrolatum for his own hair and for the hair dye. Incomplete oxidation might have been more likely to occur when the dye was not applied by an expert hairdresser.

Klein and Rodman (1981) reported vesiculation, oozing, crusting, and marked edema of the skin of the face, neck, ears, and scalp after a man dyed his own hair; his eyes were swollen shut. A patch test to p-Phenylenediamine was positive. In their review, Marzulli et al. (1978) noted that the skin around the eyes is swollen frequently in hair dye dermatitis. They commented that immediate hypersensitivity may sometimes be a component of contact dermatitis.

Edwards and Edwards (1984) reported a case of a 22-year-old beautician with acute edematous, vesicular eruption of both hands and no past history of skin disease. An intensely pruritic wheal developed within 2 minutes of application of 1% p-Phenylenediamine to the forearm. The reaction faded completely after 30 minutes. No reaction was observed at 24 hours, but a 3+ reaction was observed at 48 hours. There was no immediate or delayed hypersensitivity reaction in control patients (number not stated) patch tested with 1% p-Phenylenediamine.

LeVine (1984) reported a case of a 61-year-old male with recurrent eczematous dermatitis. Patch test (48-hour application to back) results for p-Phenylenediamine were negative. Photopatch testing was also performed. Patches were applied as usual, except that, after 24 hours, the skin was irradiated with UV-A radiation (5 joules/cm^2) from fluorescent UV-A lamps. A positive photopatch test reaction to p-Phenylenediamine was reported, and photopatch testing disclosed an idiopathic photosensitivity to UV-A. The avoidance of sunlight exposure and the use of a sunscreen prevented any further recurrence of dermatitis.

A case report by Vestey et al. (1985) involved a 25-year-old female who had a scaly, itchy eruption on the eyelids for 10 months. She had used an eyelash curler that was faced with black rubber. Patch-testing with p-Phenylenediamine mix resulted in a reaction that was classified as strongly positive.

Nethercott et al. (1986) reported 18 cases of hand dermatitis observed in hairdressers over a 5-year period. Patch test results were positive (2+ or 3+ reaction) for p-Phenylenediamine in 8 patients.

Brown et al. (1987) reported cases of 2 patients with renal failure. A 51-year-old female habitually used a henna hair dye with a p-Phenylenediamine base and a 62-year-old female had used a hair dye with a p-Phenylenediamine base for 2 years. These were described as 2 cases of vasculitis and crescentic glomerulonephritis associated with prolonged hair dye application.

Fowler (1987) patch tested a 34-year-old female with chronic, recurrent hand eczema. The patient had no history of atopy, but had an occupational history of rubber-stamping with a particular ink. Results of the 48-hour patch test revealed a ++ reaction to p-Phenylenediamine.

Goldberg et al. (1987) presented the case of a 59-year-old female, with no known history of atopy or chemical sensitivity, who had been using a variety of hair colorings for several years prior to developing evidence of hypersensitivity. The first reaction, swollen eyes and itchy hands within 10 minutes of hair dye application, occurred early in 1984. A second reaction (dysphonia, palpitations, and swollen eyes, within 20 minutes of dye application) to a hair dye from a different manufacturer was also reported. Puncture skin tests revealed a wheal and flare response to p-Phenylenediamine. The magnitude of the response was increased by adding hydrogen peroxide to p-Phenylenediamine prior to skin testing, implying reactivity to an oxidation product formed by the action of hydrogen peroxide on p-Phenylenediamine.

Estlander (1988) reported 5 cases (4 males, 1 female; 24 to 52 years old) of occupational eczema, urticaria, and respiratory disease that resulted from exposure to reactive dyes from 1977 to 1987. The patients had worked in dye houses or textile plants, and had been exposed to reactive dyes for 8 months. In 24-hour patch tests (Finn chambers), reactions were scored at the time of patch removal and at 24, 48, and 72 to 120 hours post-removal. p-Phenylenediamine (tested at 1 to 2% in petrolatum) did not induce a positive reaction in either of the 5 patients.

In a case report by Romaguera et al. (1988), a 39-year-old female presented with acute contact eczema of the foot 2 months after she began wearing an orthopedic shoe. The results of a standard patch test were positive for p-Phenylenediamine (+++ reaction).

Massone et al. (1991) presented two case reports. The first

was a 67-year-old male with relapsing erythemato-vesicular palmar dermatitis and itchy erythematous rashes on the trunk. A ++ patch test reaction to 1% p-Phenylenediamine base (in petrolatum) was observed at days 2 and 5.

In the other case report, a 50-year-old female with a history of the following reactions was patch tested with p-Phenylenediamine: allergic rhinitis, asthmatic bronchitis, costume jewelry intolerance, and an itchy skin eruption for 3 years. At day 4, a + patch test reaction to 1% p-Phenylenediamine base (in petrolatum) was reported (Massone et al. 1991).

Bork (1993) presented a case of a 35-year-old professional violinist with a five-month history of contact dermatitis on the left side of her neck. The reaction was observed weeks after using a new chin rest that was made of ebony wood. A positive patch test reaction (+++) to p-Phenylenediamine was noted at 2 days.

In a case report by Saha and Srinivas (1993), a 12-year-old patient was diagnosed with foot dermatitis, which was possibly due to the presence of p-Phenylenediamine in socks. A positive patch test reaction (+ reaction) to 1% p-Phenylenediamine in petrolatum was observed on days 2 and 3.

Lisboa et al. (1994) patch tested 6 female patients (ages between 15 and 58 years) with allergic contact dermatitis due to clothing, from January of 1988 to October of 1992. A positive patch test reaction to p-Phenylenediamine was observed in one patient.

Rebandel and Rudzki (1995) presented a case of a 51-year-old female (with no personal or family history of atopy) who had worked at a dairy plant, where she was in contact with p-Phenylenediamine daily. The results of standard patch tests indicated a striking hypersensitivity to p-Phenylenediamine.

In a case report by Pope et al. (1995), a 32-year-old male, with no significant past medical history or allergies, had a delayed localized urticarial reaction after wearing an M17 protective mask for several hours during basic combat training. Patch test results indicated a pruritic, vesicular reaction to the black rubber mix (contains p-Phenylenediamine). The patient was diagnosed with nonimmunologic contact urticaria to black rubber products containing p-Phenylenediamine.

Nakagawa and Kawai (1996) reported a case of a 62-year-old patient (no personal or family atopic history) with an itchy edematous rash on the neck and forearms, which appeared within a day of wearing a new navy-blue dress. Positive patch test reactions (++) to p-Phenylenediamine were reported on days 2 and 3.

In a case report by Fukunaga et al. (1996), syncope occurred in a 57-year-old female within minutes of using a hair dye that contained p-Phenylenediamine. Itchy bumps on the head and hands were noted 10 to 30 minutes after dye application, and these symptoms disappeared spontaneously within 3 hours. Patch tests involving the hair dye and p-Phenylenediamine indicated that the patient had an immediate-type allergy to p-Phenylenediamine. There were no signs of a delayed type allergic reaction.

Sharma et al. (1999) reported lichenoid eruptions in 4 male patients (ages 60, 32, 41, and 60) who applied black hair dye to the beard. Positive patch test reactions to p-Phenylenediamine were reported.

In a case report by Smith et al. (1999), a 49-year-old man with a two-year history of hand dermatitis had a positive patch test reaction to 1% p-Phenylenediamine.

Sahoo et al. (2000) reported a case of a non-atopic, 50-year-old clerk who experienced swelling of both eyelids 8 hours after using a hair dye. The entire face and lips also became swollen. A positive patch test reaction (+++) to p-Phenylenediamine was reported.

In a case report by Hsu et al. (2001), 8 male patients had beard dermatitis that was related to dye use. The lesions observed were described as pruritic, erythematous, papular eruptions that developed in the jaw area after each dye application. Patch test results were positive for p-Phenylenediamine.

O'Hagan and Bingham (2001) reported a case of an 11-year old school girl with a 3-year history of redness and scaling on the right thumb and index and middle finger. She had a history of infantile atopic eczema and had been playing the cello (using black-stringed bow) for a year when the rash was first observed. A positive patch test reaction (++) to p-Phenylenediamine was observed on day 4.

Önder et al. (2001) reported a case of a 9-year-old girl who had applied a temporary henna tattoo to the right arm and repeated the application 1 week later. Erythema and papulovesicular eruptions were noted at the application site. The patient had a 3+ reaction to natural henna and to 1% p-Phenylenediamine.

Shapiro et al. (2001) presented a case of a 44-year-old man (no atopic hisory) with lichenification and fissuring of the hands. While on the job, he had come in contact with dyed milk cartons that had not dried. Patch tests (Finn chambers) involved 4-day applications to the back. The last day on which reactions were scored was day 4. The only positive finding was a 2+ reaction to p-Phenylenediamine.

Simpson-Dent et al. (2001) reported a case of a 39-year-old female with a 2-year history of a pruritic rash on the lower legs (spreading to the trunk). Three months prior to presentation, she had a tattoo (black dye) applied to her arm. A positive patch test reaction (++) to p-Phenylenediamine was reported on days 2 and 4..

Ahn and Lee (2002) studied ultrastructural changes in the hair shaft following application of a permanent hair dye (containing p-Phenylenediamine, m-aminophenol, resorcin, and hydrogen peroxide) by a 26-year-old female. Scanning and transmission electron microscopy was performed immediately prior to application of the hair dye, and then at intervals ranging from 30 minutes to 8 weeks post-application. Cuticular swelling with focal degeneration was observed. Additionally, exposure of the hair cortex (due to extensive cuticular detachment) in some places along the hair fiber was also observed. Findings were most dramatic in specimens evaluated at 6 hours and day 1 post-application. Nearly complete restoration of the hair cuticle and return to the pre-coloring state were reported for the 8-week specimen.

Calzavara-Pinton et al. (2002) reported a case of a 40-year-old man with a six-month history of an eczematous eruption in major body folds. The patient was employed as a trader in oil products and often wore black synthetic materials. During patch-testing, a strong late positive reaction to p-Phenylenediamine, beginning at day 4 and lasting for as along as 4 weeks, was noted. The positive reaction was characterized by erythema and infiltration, without clinically evident vesicles.

Søsted et al. (2002) compiled data on consumer complaints relating to adverse reactions to hair dyes. Fifty-five cases (52 women, 3 men; mean age = 43 years) of severe, acute allergic contact dermatitis were reported. The main symptoms were severe edema of the face, scalp, and ears. Some of the patients (29% of the cases) were patch tested, all of whom had positive reactions to p-Phenylenediamine.

In a case report by Bhat and Smith (2003), a 38-year-old female presented with a history of discrete yellow areas on her eyelids. This was noted 6 weeks after an episode of a severe rash on her eyelids after using a black eyelash-tinting product. Patch testing with 1% p-Phenylenediamine in petrolatum resulted in a strong positive reaction, with vesiculation.

In a report by Wong and King (2003), a 42-year-old female presented with a history of a severe rash after using a hair dye. A 20-minute occluded patch test with 1% p-Phenylenediamine base in petrolatum resulted in an immediate urticarial reaction at the test site on the right ventral forearm. An eczematous reaction developed at this site 48 hours later.

Søsted et al. (2004) presented cases of 9 patients (8 females, 1 male) who had dermatitis that was related to the use of oxidative hair dyes. Each patient used a different hair dye product, and the 9 products represented a total of 4 international cosmetic manufacturers. The females ranged in age from 20 to 58 years, and the male was 21 years old. Patch test reactions to p-Phenylenediamine were positive (+ reaction or greater) in all of the 9 patients who were tested.

In a case report by Charles et al. (2004), a positive patch test reaction to p-Phenylenediamine was reported in a 40-year-old female (former hairdresser) with ulcerative colitis.

Lim et al. (2004) presented a case of a 9-year-old girl (no history of atopy) who had a henna tattoo applied to her back. Within 48 hours of tattoo application, acute allergic contact dermatitis (erythema, edema, and vesiculation) was observed. A +++ patch test reaction to p-Phenylenediamine was reported.

In a case report by Ho et al. (2004b), acute dermatitis on the scalp, ears, pre-auricular areas and neck was observed in a 40-year-old male one day after dyeing his hair. Sensitization to p-Phenylenediamine in the dye was suspected. Patch test results revealed a +++ reaction to 1% p-Phenylenediamine in petrolatum. Cross-reactions (+ reactions) to Disperse Orange 3 and Disperse Red 17, at a test concentration of 1% in petrolatum, were also reported.

Saunders et al. (2004) presented a case of a 28-year-old mill worker at an aluminum refinery who presented with a 10-month history of an erythematous and pruritic rash on the dorsa of her feet, extending to the ankles. The patient developed an itchy rash at the site of application of a temporary tattoo 2.5 years ago and had dyed her hair 5 years ago. Patch tests (Finn chambers on Scanpor tape) were performed and reactions scored accoring to ICDRG recommendatiions on days 2 and 5. A positive (2+) reaction to p-Phenylenediamine was reported.

In case reports by Matulich and Sullivan (2005), 2 female subjects (15 and 17 years) each had a temporary black henna tattoo applied to the lower back, and a repeat application of the mixture was made 2 days later. The 17-year-old subject (history of hay fever and first-degree family history of atopy) developed a pruritic vesicular reaction in the area of the tattoo at day 10 after the first application. The reaction developed into a vesico-pustular reaction, which spread rapidly to the feet, thighs, axillae, arms, trunk, face, and scalp.

The 15-year-old subject (history of eczema as young child) developed a pruritic vesicular eruption at the site of the tattoo, which remained localized. Patch tests were conducted using Finn chambers on Scanpor tape. The patches remained in place for 48 hours, and reactions were scored at 72 hours and at 7 days.

For the 17-year-old subject, patch-testing confirmed an extreme, spreading vesicobullous reaction (strongly positive) to p-Phenylenediamine. Patch test results for the 15-year-old subject confirmed a weak reaction to p-Phenylenediamine (Matulich and Sullivan 2005).

Søsted and Menné (2005) patch-tested a 50-year-old female with a history of nickel allergy. The patient had dyed her hair and developed a scalp dermatitis with severe itching

that had spread to her face, neck, and upper thorax. Patch tests were conducted using Finn chambers on Scanpor tape, and a weakly positive reaction (+?) to p-Phenylenediamine (1% in petrolatum) at days 3 and 7 was reported.

Martin et al. (2005a) patch-tested a 14-year-old female who presented with an acutely inflamed eruption that was described as exudative and eczematous. The eruption involved the entire scalp, posterior neck, ears, and forehead. The acute scalp dermatitis was thought to have been an allergic contact dermatitis reaction to p-Phenylenediamine in the hair dyes. Erythema and edema of the left side of the face and periorbital region were observed. Signs were observed within hours of applying 2 permanent hair dyes at home 2 days ago. It is also important to note that the patient had a temporary 'black henna tattoo' applied to the right upper arm 3 years ago. Patch test results indicated a positive (+++), necrotic reaction to 1% p-Phenylenediamine.

In a study by Jasim et al. (2005), 2 cases were presented. A 15-year-old boy presented with facial redness after his hair had been dyed (permanent hair dye bleached). On the following morning, his entire face and neck were swollen. It is important to note that a blistering reaction to a black henna tattoo occurred a year ago. When the subject was tested with p-Phenylenediamine (20-minute application), severe blistering was observed at 24 hours.

A 14-year-old girl presented with severe facial swelling and redness a few hours after having her hair dyed. She also reported a reaction to henna tattoos that had occurred 2 years previously. When the subject was tested with p-Phenylenediamine (20-minute application), a strong reaction was observed at 4 hours (Jasim et al., 2005).

In a study by Søsted et al. (2006b), 8 children (between 12 and 15 years old) suspected of having an allergic reaction to a hair dye product were referred to dermatology clinics located in Denmark. For 5 of the patients, hair coloring had been performed at home. For the remaining 3 patients, hair dyeing was performed by a hairdresser. Six patients had a history of skin reactions to a temporary tattoo and 2 had reactions to hair dyes only. Patch tests were performed according to ICDRG recommendations. Occlusive patches were applied to the upper back for 2 days, and reactions were scored at day 3 and/or day 4 and day 7. Patch test results indicated positive reactions to p-Phenylenediamine in all subjects (++, 5 subjects; +++, 3 subjects).

Paley et al. (2006) reported a case of a 67-year-old male with a complaint of itchy, red bumps on the face and scalp. Symptoms were observed approximately 1 month before the initial visit to a dermatologist. At physical examination, there were erythematous nodules and tumors without epidermal changes on the right temple and postauricular area. A biopsy revealed a dense, nodular dermal infiltrate that consisted predominantly of lymphocytes along with some eosinophils and histiocytes. Prior to the visit, the patient had been coloring his hair regularly for over 5 years. After considering the location of the lesions, the history of exposure, and the characteristic pathology, the diagnosis of pseudolymphoma secondary to hair cosmetic allergy was suspected. Patch test results indicated a 2+ reaction to p-Phenylenediamine and cinnamic alcohol and a 1+ reaction to fragrance mix, and the patient was instructed to avoid p-Phenylenediamine. Use of a p-Phenylenediamine-free hair dye was recommended. At a 2-month follow-up visit, it was noted that all lesions had resolved.

In a case report by Teixeira et al. (2006), a 30-year-old female atopic patient developed a severe dermatitis, on the eyelids and periorbital region, and conjunctivitis after her eyelashes were tinted with a permanent black eyelash and eyebrow dye. The dye was tested in a semiocclusive patch test. Patches were secured with Miocropore® tape, and reactions were scored after 2 and 4 days. Positive patch test reactions to the eyelash dye and to p-Phenylenediamine (0.01% and 1% in petrolatum) were observed.

Birnie and English (2007) reported a case of a 17-year-old hairdresser trainee whose arm became red and extremely pruritic after testing with hair dyes. The patient was scratch tested with 3 hair dyes, 2 of which contained p-Phenylenediamine. Within 10 minutes, a strong urticarial reaction to the two hair dyes containing p-Phenylenediamine was observed. A positive urticarial reaction was also observed following testing with 1% p-Phenylenediamine in petrolatum. A diagnosis of immediate hypersensitivity (type 1 allergy) to p-Phenylenediamine, resulting in urticaria, was made.

Following the ingestion of p-Phenylenediamine (7 g) by a pregnant female (Younous et al. 2007), a uterine ultrasound showed normal fetus viability, normal heart noises, a normally inserted placenta, and biometry that was consistent with the end of pregnancy. The clinical and biological assessments (creatine phosphokinase [CPK], creatinemia, and kalemia) of the child immediately after delivery by Cesarean section did not show any of the classic signs of p-Phenylenediamine poisoning. The authors noted that this case report highlights the fact that p-Phenylenediamine does not seem to cross the placental barrier.

p-Phenylenediamine HCl

According to Su and Sun (1998), a 47-year-old female hairdresser with a 4-year history of itchy erythema over the hands was patch-tested. The patient had neither a family nor personal history of atopy. A positive (++) patch test reaction to p-Phenylenediamine HCl (test concentration not stated) was reported on days 2 and 4.

In a case report by Galindo et al. (1994), a 23-year-old male who worked at a photographic developing laboratory for 2 years presented with eczematous lesions on the hands, wrists, and forearms. The lesions were first observed after

the first month of employment. Patch test results for 1% p-Phenylenediamine HCl in petrolatum were negative on days 2 and 4. Patch test reactions for the ten control subjects were also negative.

Case Reports Related to Tattooing

As noted earlier, the U.S. FDA has determined that uses of p-Phenylenediamine other than as a hair dye are unapproved, including as one of the additives that is used to accelerate drying and darken the reddish color of the henna in dark (black) henna products. Such use apparently continues, however, based on the case reports below.

p-Phenylenediamine

In a case report by Santucci et al. (1994), hypertrophic allergic contact dermatitis was observed in a 26-year-old female following application of a black hair dye to the skin. The patient was patch-tested with 0.5% p-Phenylenediamine (in petrolatum) using Finn chambers. Reactions were scored at 2 and 3 days, and a papulovesicular reaction to 0.5% p-Phenylenediamine was reported.

Wakelin et al. (1998) reported a case of a 32-year-old female who developed an acute blistering eruption on the dorsum of the hands, forearms, and feet 2 weeks after application of a black hair dye containing p-Phenylenediamine to the skin. Patch test results revealed ++ and +++ reactions to a 1% p-Phenylenediamine base in petrolatum at days 2 and 4, respectively.

Gallo et al. (1999) presented a case of a 26-year-old female who had a design (henna dye used) painted on her right shoulder. Patch test reactions to 1% p-Phenylenediamine (in petrolatum) were ++ on days 2 and 3.

Tosti et al. (2000) presented cases of 3 patients with allergic contact dermatitis following the application of a temporary henna tattoo. The first patient (30 years old) had a ++ reaction to 1% p-Phenylenediamine (in petrolatum) on days 2 and 3. The second patient (36 years old) had a positive reaction to 1% p-Phenylenediamine in petrolatum. Patch test results for the third patient (48 years old) were positive (+++ reaction) for p-Phenylenediamine on days 2 and 3.

In a report by Sidbury and Storrs (2000), a 35-year-old male presented with a circumferential, erythematous, vesicular eruption on the right, proximal upper extremity, the site of application of a henna tattoo. The patient had a past medical history of respiratory atopy, but no known personal history of exposure to henna or hair dyes. A positive (3+) patch test reaction to 1% p-Phenylenediamine in petrolatum was reported.

Mohamed and Nixon (2000) reported swelling and scabbing at 7 to 8 days after application of 2 temporary tattoos to both arms of a 20-year-old male. The skin paint contained 2.94% p-Phenylenediamine. Patch test results revealed a very strong (3+) reaction to p-Phenylenediamine. Two temporary tattoos were also applied to the left upper arm of a 42-year-old female with 2 temporary tattoos on left upper arm. Within 48 h after application of the second tattoo, itching, weeping and a crusted area were observed. Patch testing revealed a 3+ reaction to p-Phenylenediamine.

In a case report by LeCoz et al. (2000), 4 patients (7, 8, 20, and 25 years old) developed allergic contact dermatitis after their skin was painted with black henna. For the 3 patients patch tested, results were positive for p-Phenylenediamine. The patch test procedure was described as follows: patches (1% p-Phenylenediamine in white petrolatum, Finn chambers) remained in place for 48 hours and reactions were scored at 48- and 72-hour readings.

Nikkels et al. (2000) reported a case of a 10-year-old girl who had a skin decoration (henna dye) painted on the right arm. Upon returning home, she developed an allergic contact dermatitis that was restricted to the site of skin decoration. The patient had no history of contact dermatitis, but suffered from hay fever. Patch testing revealed a severe (+++) reaction to p-Phenylenediamine. The same severe reaction was observed in 2 other cases (17-year-old boy, 8-year-old girl) after a skin decoration (henna dye) was applied to the neck and arm, respectively.

In a case report by Chung et al. (2001), 4 patients developed itchy and inflamed skin reactions to temporary paint-on tattoos (vegetable dye or henna). All developed intense itchy, raised or swollen and erythematous eruptions along the tattoo designs at 10 to 14 days post-application. Patch tests were removed after 48 hours, and reactions were scored according to ICDRG recommendations at 72 hours. Patch test results for case 1 indicated a +3 reaction to p-Phenylenediamine and 2 +1 reactions to 10% and 20% aqueous henna powder, respectively. Patch test results for case 2, case 3, and case 4 indicated +3, +2, and +3 reactions to p-Phenylenediamine, respectively.

In a case report by Kulkarni et al. (2001), a 2+ patch test reaction (with vesiculation) to p-Phenylenediamine was reported for a 26-year-old male who had received a black henna tattoo.

In a case report by Läuchli et al. (2001), 4 patients (31, 32, 33, and 43 years, respectively) developed contact dermatitis to black henna tattoos. At 2 to 10 days after skin painting, itching, erythema, and swelling at the application site were reported. Microscopic examination revealed spongiotic dermatitis with dense lymphohistiocytic infiltrates. At 72 hours, a strongly positive patch test reaction to p-Phenylenediamine (in petrolatum) was reported.

Avnstorp et al. (2002) presented a case of a 24-year-old male with a vesiculobullous reaction on the fingertips. Four days prior to the eruption, the patient had used his fingertips to paint a subject with temporary tattoo paint. He also had a temporary tattoo applied to the right side of his neck four

years ago. The temporary tattoo paint was found to contain 0.3 % w/w p-Phenylenediamine. Patch test (Finn chamber on Scanpor tape) results indicated a +++ reaction to p-Phenylenediamine.

Brancaccio et al. (2002) presented a case of a 37-year-old female with black henna tattoos on the left upper arm and lower back. Within 24 to 48 hours, pruritic dermatitis was observed at both sites. The patient was patch tested (Finn chambers) with 1% p-Phenylenediamine in petrolatum. Within 7 hours, the test sites became severely pruritic. At 24 hours post-application, a strongly positive 3+ (erythema, edema, and vesicles) reaction was observed at both test sites. By 1 week, the reaction persisted and remained strongly positive. Reactions were not observed in any of the 10 control patients.

According to Chung et al. (2002), 10 patients (18 to 28 years old) developed inflamed skin eruptions after receiving temporary paint-on tattoos. The 6 patients who were patch-tested (48-hour IQ chamber; reactions scored at 72 hours) all had moderately to strongly positive reactions to p-Phenylenediamine..

Marcoux et al. (2002) reported a case of a 17-year-old female with an acute, itchy eruption over the ears, temples, and scalp. The eruption was accompanied by severe edema of the periorbital, perimandibular, and anterocervical soft tissues. Complaints occurred 12 hours after coloring the hair at home, and, also, a henna tattoo was applied to the right shoulder 7 months earlier. The patients' past medical history included atopic dermatitis during childhood and hyperthyroidism (diagnosed at 13 years of age). Patch test results for 1% p-Phenylenediamine were positive (3+ reaction).

Neri et al. (2002) presented 2 case reports. The first case was a 9-year-old boy with an erythema multiforme-like eruption, mainly localized on the limbs, for 4 days. He also had an eczematous, itchy patch at the application site of a henna tattoo. The boy's personal medical history was unremarkable with respect to atopy or allergic reactions. Patch test results at 48 and 72 hours revealed a 3+ reaction to p-Phenylenediamine in petrolatum. The second case was a 7-year-old girl with an eczematous reaction at the appplication site of a henna tattoo. Erythema, swelling, and vesicles, accompanied by itching, were reported. Patch test results for 1% p-Phenylenediamine (in petrolatum) were negative at 48 and 72 hours.

According to Pegas et al. (2002), a 12-year-old boy received a henna tattoo, and application was followed by severe itching and 2 erythematous, papulous lesions at the tattoo site. A skin biopsy revealed a lymphomononuclear interstitial and perivascular dermal infiltrate, with follicular epidermal spongiosis. Patch test (Finn chamber) results for p-Phenylenediamine were positive (+++).

Van Zuuren and Lavrijsen (2002) reported cases of 3 patients (8- year-old boy, 10- year-old boy, and 30-year-old female) with allergic contact dermatitis and hypopigmentation after application of a henna tattoo. A strongly positive patch test reaction to p-Phenylenediamine was reported for all three patients.

Nawaf et al. (2003) reported a case of a 17-year-old girl with blisters over her hands over a period of 5 days. The blisters were first observed within 72 hours of applying a temporary henna paint to the hands. Similar lesions were observed on the face. A clinical diagnosis of acute allergic contact dermatitis was made. Patch testing of the patient with 1% p-Phenylenediamine in petrolatum (48-hour application) yielded a 3+ reaction. No reaction was observed at the site patch tested with natural henna powder.

In a case report by Wolf et al. (2003), 6 patients [ages: 11 (male), 11 (male), 18 (male), 18 (female), 17 (female), and 12 (female)] developed allergic contact dermatitis after skin painting with black henna. These patients also showed hypersensitivity to p-Phenylenediamine (2+ to 3+ reactions).

In a report by Ho et al. (2004a), 12 (11 females; 1 male; ages: 6 to 54 years old) patients at a contact dermatitis clinic had positive patch test reactions to p-Phenylenediamine, and had a prior history of exposure to temporary black henna tattoos between April of 1997 and April of 2004. Except for one of the patients (20-minute patch application), the patches remained in place for 2 days. Patch test results were as follows: ++ or +++ reaction to 1% p-Phenylenediamine in petrolatum (6 patients); +++ reaction to 0.3% p-Phenylenediamine in petrolatum (1 patient); + or ++ reaction to 0.01% p-Phenylenediamine in petrolatum; and + reaction to 1% p-Phenylenediamine in petrolatum (1 patient, after 20 minute application).

In a case report by Blair et al. (2004), a 32-year-old female presented with a 5-day history of a severely pruritic, erythematous reaction at the site of a temporary black henna tattoo. The tattoo had been applied 2 weeks earlier. The patient had no history of prior exposure to henna in hair dyes or coloring her hair. Patch test results indicated a 2+ reaction to p-Phenylenediamine.

Borrego et al. (2005) patch-tested a 24-year-old female who presented with chronic dermatitis on the hands, during the previous year, which became severe during employment as a temporary tattoo artisan. An inflammatory skin reaction was observed when, on 1 occasion, she applied a temporary tattoo to herself. A positive patch test reaction to p-Phenylenediamine (+++) was observed at 48 and 96 hours.

Martin et al. (2005b) presented 3 cases (6, 29, and 41 years old) of allergic contact dermatitis to temporary henna tattoos. p-Phenylenediamine is 1 of the additives that is used to accelerate drying and darken the reddish color of the

henna. Patch test results for p-Phenylenediamine were positive in 2 of the patients. In 1 of the 2 patients, the lesions resolved, leaving persistent hypopigmentation.

In a case report by Tomlijanović-Veselski (2006), an 11-year-old boy experienced a burning sensation and marked redness at the site of tattoo application (right brachium) on day 1 after application of the tattoo. On day 10, the patient visited a dermatologist because of persistent lesions, with pronounced redness and scaling at the application site. Visible residual hypopigmentation was present at 4 weeks after application of the tattoo. Patch test results were positive for p-Phenylenediamine. The patch test concentration of p-Phenylenediamine (0.5%) was said to have been10 times lower than the concentration of p-Phenylenediamine in the tattoo paste. The authors noted that the severe reaction on the day following application of the tattoo could be explained by sensitization to a p-Phenylenediamine-containing tattoo the year before.

Jung et al. (2006a) reported a case of a 9-year-old boy who experienced a painful, itchy blistering reaction 2 weeks after being painted with a henna tattoo. The reaction spread to the arms, trunk, and feet. The boy's hair was dyed black 6 months later and papulovesicular eruptions at the hairline, accompanied by pruritus, were observed the next morning. A positive (+++) patch test reaction to 0.2% p-Phenylenediamine was reported.

In a case report by Jung et al. (2006b), exudative bullous eruptions (painful and itchy) were observed on a 40-year-old male several hours after he had a henna tattoo painted on his left upper arm. Patch test results indicated a +++ reaction to p-Phenylenediamine. The patient's past medical history (4 years earlier) revealed erythematous, vesiculous dermatitis in tattooed areas 2 hours after the application of a temporary henna tattoo. Post-inflammatory hypopigmentation, in the design of the original tattoo, persisted for 1 year after the lesions had resolved.

Tan and Garioch (2007) reported a case of an 8-year-old boy with a 3-day history of an itchy vesicular rash at the site of a temporary henna tattoo that had been obtained one week ago. The boy was diagnosed with contact dermatitis to black henna tattoo with auto-eczematization. Positive patch test reactions to 1% p-Phenylenediamine in petrolatum were reported (+++ at 48h and 96h).

Skin Depigmentation

p-Phenylenediamine

Taylor et al. (1993) presented a case of a 67-year-old male who used hair dyes and had a 4.5 year history of depigmentation of the scalp and forehead. A positive patch test reaction (2+) to 5% aqueous p-Phenylenediamine was noted at 72 hours, and the patch test site was completely depigmented at 2 years post-application. Skin depigmentation was also observed in 3 other males (62, 45, and 56 years old) who had used hair dyes.

Brancaccio and Cohen (1995) reported a case of a 51-year-old male, with no history of vitiligo, who developed contact leukoderma after using a mustache coloring solution. At week 4, a physical examination revealed complete depigmentation of approximately 50% of the mustache area and 4 satellite areas of depigmentation on the face and neck. Patch test results for p-Phenylenediamine were negative at 2 and 7 days. No pigmentary alterations were noted at patch test sites for up to 2 weeks after testing.

In a case report by Bajaj et al. (1996), a 55-year-old female applied a hair dye containing 16% p-Phenylenediamine for 3 hours. Itching and burning over the entire scalp were reported after 8 to 10 applications of this type. Within 24 hours, exudation and edema over the scalp, which subsided with time, were observed. Depigmentation of the scalp was noted 3 to 4 weeks later. At 1.5 years after the onset of depigmentation (hair dye not used for 1.5 years), depigmentation was noted over the scalp, back of neck, and forehead.

In a case report by Bajaj et al. (1998), a 50-year-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. Itching and scaling were also reported. Patch testing (48-hour patch) with 1% p-Phenylenediamine (in plastibase) resulted in a + reaction and depigmentation at the application site.

In a case report by Bajaj et al. 2000, a 34-year-old female presented with depigmentation (over a 4- month period) on the sides and dorsa of both feet, where alta (red skin decoration) had been applied. Itching and dermatitis were also reported. During the patch test, patches were removed on day 2 and reactions were scored on days 2 and 3. A positive reaction (+ reaction) to p-Phenylenediamine was reported.

Jappe et al. (2001) reported cases of 5 youths (9 to 17 years old) with a paint-on henna tattoo on the arm. An erythema-multiforme-like eruption and depigmentation were observed following an allergic contact dermatitis reaction to the tattoo. Patch test results for p-Phenylenediamine were strongly positive in all patients.

HAIR DYE EPIDEMIOLOGY

Hair dyes may be broadly grouped into oxidative (permanent) and direct (semipermanent) hair dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes are a preformed color. p-Phenylenediamine, p-Phenylenediamine HCl, and p-Phenylenediamine Sulfate are oxidative/ permanent dyes. While the safety of individual hair dye ingredients are not addressed in epidemiology studies that seek to determine

links, if any, between hair dye use and disease, such studies do provide broad information and have been considered by the CIR Expert Panel.

In 1993, an International Agency for Research on Cancer (IARC) working group evaluated 78 epidemiology literature citations and concluded that "personal use of hair colourants cannot be evaluated as to its carcinogenicity" and that "occupation as a hairdresser or barber entails exposures that are probably carcinogenic" (IARC, 1993). The IARC report did not distinguish between personal use of oxidative/permanent versus direct hair dyes, or distinguish among the multiple chemical exposures in addition to hair dyes to which a hairdresser or barber might be exposed.

Rollison et al. (2006) reviewed the available epidemiology literature published from 1992 through February 2005, which includes over 80 citations on personal hair dye use published since the IARC review. The authors found that hair dye exposure assessment ranged from ever/never use to information on type, color, duration and frequency of use. The authors found insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. The review highlighted well-designed studies with an exposure assessment that included hair dye type, color, and frequency or duration of use, which found associations between personal hair dye use and development of acute leukemia, bladder cancer, multiple myeloma, and non-Hodgkin's lymphoma. These findings, however, were not consistently observed across studies.

Several studies addressing the possible link between hair dye use and bladder cancer, lymphoma and leukemia, other cancers, reproductive and developmental outcomes, and other endpoints published since the above review also have been considered. A summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml.

SUMMARY

p-Phenylenediamine is an aromatic amine that has been used in permanent hair dyes for over 100 years. It is mixed with hydrogen peroxide immediately before use, and the resulting oxidation products either react with sulfhydryl groups present in hair to form permanent bonds or are simply trapped in hair protein. Data provided to FDA by industry as part of the Voluntary Cosmetic Reporting Program indicated that p-Phenylenediamine was used in a total of 1497 hair-coloring products; there were no reported uses of p-Phenylenediamine HCl or p-Phenylenedianmine Sulfate. Use concentration data provided by the Cosmetic, Toiletry, and Fragrance Associaton indicated that p-Phenylenediamine was being used in cosmetics at concentrations ranging from 2% to 4% and that both p-Phenylenediamine HCl and p-Phenylenediamine Sulfate were being used at concentrations of 6%.

By Federal law, coal tar hair dye products, including those containing p-Phenylenediamine, are exempt from the principal adulteration provision and the color additive provisions of the Federal Food, Drug and Cosmetic Act of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation.

The U.S. FDA has determined that uses of p-Phenylenediamine other than as a hair dye are unapproved, including as one of the additives that is used to accelerate drying and darken the reddish color of the henna in dark (black) henna products.

p-Phenylenediamine is manufactured using the following three methods: (1) reduction of para-nitroaniline, (2) aniline diazotization, and (3) direct nitration of benzene without chlorinating. The third method does not yield chlorinated compounds such as chloro- and dichloroanilines or aminobiphenyls.

p-Phenylenediamine has a low octanol/water coefficient. Following dermal administration of radiolabeled p-Phenylenediamine to rats, the metabolite N,N'-diacetyl-p-Phenylenediamine was detected in the plasma, suggesting that topically applied p-Phenylenediamine is metabolized in the skin. p-Phenylenediamine administered orally, intraperitoneally, or subcutaneously to mice, rats, and rabbits was widely distributed, metabolized to several derivatives, and rapidly excreted in the urine and feces.

p-Phenylenediamine applied topically to humans, was found in the urine. When a hair dye containing p-Phenylenediamine was used by humans, radioactivity was detected in the hair and in the urine. In human studies, monoacetyl-p-Phenylenediamine (MAPPD) and N,N'-diacetyl-p-Phenylenediamine (DAPPD) have been identified as urinary metabolites of p-Phenylenediamine. The results of another human in vivo study of an oxidative hair dye formulation containing p-Phenylenediamine HCl suggested that dyeing the hair with oxidative dyes produces minimal systemic exposure that is unlikely to pose a risk to human health.

The acute oral LD_{50} of p-Phenylenediamine for rats ranged from 80 to 98 mg/kg. Dietary p-Phenylenediamine at concentrations of 3160 ppm administered to rats and 4640 ppm to mice for 7 weeks and 1250 ppm to rats and mice for 103 weeks did not result in any signs of toxicity. In another study, dose-dependent growth retardation was observed in rats fed concentrations up to 0.4% p-Phenylenediamine in the diet for 12 weeks. 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. lntraperitoneal 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.

No signs of toxicity were observed when dry p-Phenylenediamine or a 10% alcoholic solution of p-Phenylenediamine was applied to a 25 cm^2 area of the skin of rabbits.

The percutaneous LD_{50} (for rabbits) of a hair dye composite containing 1.2% p-Phenylenediamine was greater than 10 g/kg. Chronic topical administration of 10% p-Phenylenediamine solutions was not toxic to mice or rabbits. Subchronic and chronic dermal administration of hair dye products containing up to 4% p-Phenylenediamine was not toxic to mice, rabbits, and rats. In guinea pigs, evidence of hyperkeratosis was noted at 24 h post-application (under occlusive patch) of p-Phenylenediamine. At 48 h, discontinuity appeared in the stratum germinativum; a moderate degree of edema was observed in the dermis.

At single oral doses up to 80 mg/kg, p-Phenylenediamine did not exert a primary effect on the nervous system of rats. Rhabdomyolysis was observed in mice dosed orally with 35 mg/kg and 70 mg/kg p-Phenylenediamine. Rhabdomyolysis was also observed in dogs at doses up to 100 mg/kg p-Phenylenediamine and massive necrosis of skeletal muscles was observed at microscopic examination in these animals.

Necrosis of the gastrocnemius muscle, diaphragm, and tongue were observed at a dose of 200 µmol/kg/day, but not 100 µmol/kg/day in rats injected subcutaneously with p-Phenylenediamine daily for 3 days.

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. 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. In one study, p-Phenylenediamine was a guinea pig sensitizer at induction concentrations as low as 0.001%, but was not a sensitizer in another study of a haircoloring formulation containing 2% p-Phenylenediamine.

Cross-reactivities to p-Phenylenediamine were confirmed in guinea pigs challenged with p-toluenediamine ·HCl, p-aminophenol, p-aminoazobenzene, and Sudan III in the maximization test. When the cross-reactivity of p-Phenylenediamine HCl with color developing agents (p-Phenylenediamine derivatives) was evaluated using the maximization test procedure, there was no cross reactivity between p-Phenylenediamine and 4-N,N-diethyl-2-methyl-1,4-phenylenediamine · HCl (CD-2) or 4-(N-ethyl-N-2-methan-sulphonamidoethyl)-2-methyl-1,4-phenylenediamine · 1.5 H_2SO_4 · H_2O (CD-3).

Mild conjunctival inflammation that did not persist for more than 24 hours was observed after the instillation of a 2.5% aqueous p-Phenylenediamine solution into rabbit eyes. 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 day postinstillation, 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 days.

Doses of 5 to 30 mg/kg per day of p-Phenylenediamine by gavage to pregnant rats did not affect reproduction, and p-Phenylenediamine was not teratogenic. Oral administration of 40 mg/kg p-Phenylenediamine to pregnant rats resulted in maternal toxicity. The maternal no-observed-effect level (NOEL) was 5 mg/kg/day and the developmental no-observed-adverse effect level (NOAEL) was 10 mg/kg/day. Subcutaneous administration of 28 mg/kg per day p-Phenylenediamine to pregnant mice did not result in embryotoxic or teratogenic effects. No evidence of an increase in postimplantation fetal loss occurred when male rats received 2 to 20 mg/kg p-Phenylenediamine intraperitoneally 3 times a week for 8 weeks and then were mated. The results of a chick embryotoxicity screening test indicated that p-Phenylenediamine Sulfate induced significant malformations of the body wall and heart.

Hair dyes containing 1.0 to 4.0% p-Phenylenediamine applied to the skin of pregnant rats at a dose of 2 ml/kg per day after being mixed with an equal volume of hydrogen peroxide produced no adverse effects on reproduction and were not teratogenic. A hair dye containing 3% p-Phenylenediamine mixed with hydrogen peroxide, applied dermally 2 times a week to female mice prior to mating and throughout gestation, resulted in no adverse effects on reproduction and was not teratogenic, although there may have been a retarding effect on fetal ossification. The same hair dye containing 3% p-Phenylenediamine applied dermally at a dose of 2.0 ml/kg 2 times a week to female rabbits prior to mating and through gestation produced no adverse effects on rabbit reproduction, and the dye was not teratogenic. 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 and mixed with hydrogen peroxide 2 times a week to 3 generations of mice. A hair dye containing 2.20% p-Phenylenediamine applied to the skin of male rats in a dose of 0.5 ml 2 times a week for 10 weeks, after being mixed with an equal volume of hydrogen peroxide, produced no adverse effects on reproduction.

p-Phenylenediamine, with or without hydrogen peroxide, was negative in the Ames Salmonella/mammalian-microsome mutagenicity test without metabolic activation.

Both positive and negative results with metabolic activation have been reported. Different researchers have used different solvents for the p-Phenylenediamine, different chemicals for induction, different S-9s, and slight modifications to the Ames test procedure. Any or all of these may explain the observed differences in results. Several oxidation products of p-Phenylenediamine were positive in the Ames test. Purified p-Phenylenediamine was not mutagenic to *D. melanogaster*. p-Phenylenediamine was not mutagenic in the rat or mouse micronucleus test. 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 to 20 mg/kg per day for 5 days 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.

The urine of rats that received p-Phenylenediamine intraperitoneally 3 times a week for 8 weeks was not mutagenic in the Ames test. The urine of rats that received p-Phenylenediamine/resorcinol conjugates topically was mutagenic with metabolic activation and was not mutagenic without metabolic activation. The urine from women who used hair dyes containing 0.46 to 2.55% p-Phenylenediamine was not mutagenic with metabolic activation in the Ames test.

p-Phenylenediamine HCl was mutagenic to one (strain TA98) of five *Salmonella typhimurium* strains tested with metabolic activation. Both positive and negative results (with and without metabolic activation) were reported for p-Phenylenediamine HCl in the L5178Y mouse lymphoma assay. In the TK6 human lymphoblast forward mutation assay, results for p-Phenylenediamine HCl were positive without, but not with, metabolic activation. Except for the Chinese hamster ovary cell/hypoxanthine-guanine phosphoribosyl transferase (CHO/HGPRT) mutation assay (positive without, but not with, metabolic activation), the results for p-Phenylenediamine HCl in other mammalian assays (micronucleus, replicative DNA synthesis, and DNA damage) were negative. Mixtures of p-Phenylenediamine, HCl, resorcinol, and hydrogen peroxide were not mutagenic in the mouse lymphoma assay or the chromosome aberrations assay (human lymphocytes).

In *Salmonella typhimurium* strain TA98, the mutagenicity of p-Phenylenediamine HCl was enhanced by H_2O_2 treatment in the presence of metabolic activation. p-Phenylenediamine HCl had no effect on the gene expression profile of a monocytic leukemia cell line (THP-1). In a DNA binding study, there was no evidence of covalent binding of p-Phenylenediamine HCl or metabolites with rat hepatic DNA.

p-Phenylenediamine in the feed of rats and mice at concentrations of 625 and 1250 ppm for 103 weeks was not carcinogenic. There was no evidence of a carcinogenic effect after the oral administration of 0.06 to 30 mg/kg per day p-Phenylenediamine for 8 months to rats. Results were also negative for p-Phenylenediamine in an 80-week oral carcinogenicity study involving rats. p-Phenylenediamine 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 weeks. It also did not modify liver carcinogenesis in male rats initially treated with N-nitrosodiethylamine. The results for p-Phenylenediamine HCl were classified as negative in a study evaluating its potential for the induction of lung tumors in mice. In female rats, both topical application and s.c. injection of oxidized p-Phenylenediamine HCl for 18 months induced a statistically significant increased incidence of mammary gland tumors. Uterine tumors and soft tissue tumors of both malignant and benign types were also significantly induced. Tumors of the mammary gland and soft tissue were not observed in male rats.

When the carcinogenic potential of p-Phenylenediamine HCl was evaluated in a bioassay system that was based on the induction of glutathione S-transferase placental form (GST-P) positive liver cell foci in rats, unlike the 5 genotoxic hepatocarcinogens evaluated, this hair dye did not cause a significant increase in the number of GST-P positive foci.

Three hair dyes containing 1.5% p-Phenylenediamine mixed with hydrogen peroxide before use, and applied topically to mice weekly or every 2 weeks for 18 months were not carcinogenic. No evidence of a carcinogenic effect was found after the topical administration (0.5 ml weekly for 2 years) 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 to mice weekly for 21 to 23 months. 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 2 times a week for 2 years. An increase in pituitary adenomas was observed in the rats receiving the 4% formulation; these adenomas have a high background incidence in rats. A non-significant pattern for this pituitary tumor was observed in the two lower dose groups.

The PII for 50% p-Phenylenediamine applied to the skin of 6 human volunteers for 24 hours under occlusive conditions was 0.8 of a maximum possible total of 8. All of 24 subjects were sensitized after 5 48-hour induction patches of 10% p-Phenylenediamine. Positive (urticarial) scratch test reactions to p-Phenylenediamine mix (black rubber mix)

were observed in 2 of 31 patients with contact urticaria and/or systemic reactions to different latex products. In another study, a positive immediate patch test reaction (at 30 minutes post application) was observed in 2 of 664 patients patch tested with p-Phenylenediamine.

p-Phenylenediamine tested at concentrations ranging from 1% to 10% in predictive patch tests induced sensitization in each of the 24 subjects tested at the highest test concentration (maximization test). The following positive reactions were reported for 2171 subjects tested with 1% p-Phenylenediamine in petrolatum in a 48 h occlusive patch test: 80 positives in 1017 subjects who used a low p-Phenylenediamine concentration hair dye product frequently; 7 positives in 548 subjects who used a hair dye with twice the p-Phenylenediamine concentration, but less frequently; and 2 positives in 516 subjects who did not use p-Phenylenediamine hair dyes.

Patch test results for 80 chronic hemodialysis patients indicated 14 patients with reactions to various substances, 3 of whom had a positive reaction to 3.75% p-Phenylenediamine. Fifteen patients (allergic to p-Phenylenediamine) were patch tested with 1% p-Phenylenediamine and serial dilutions down to 1 ppm. Of the 15, 14 had a weakly positive reaction to 1% p-Phenylenediamine. The threshold value for 10% of the patients tested, based on + reactions or greater on the back, was 38 ppm. Of 13,300 dermatitis patients patch tested with 1% p-Phenylenediamine in petrolatum from 1990 to 2000, 449 positive reactions were reported.

Over a 9-year period, 42,839 dermatitis patients were patch tested with both dyes (p-Phenylenediamine HCl, from 1984 to 1988; p-Phenylenediamine, from 1989 to 1993) and 1481 positive reactions to p-Phenylenediamine (data on both dyes combined) were reported. In another study, 26,706 dermatitis patients were patch tested with 0.5% p-Phenylenediamine HCl (between 1985 and 1988) and 0.5% p-Phenylenediamine (from 1989 to 1998) and 667 positive reactions to p-Phenylenediamine (data on both dyes combined) were reported. The results of other provocative tests indicated that the incidence of positive patch test reactions to 2 hair dyes, after mixing with hydrogen peroxide, varied from 15.4 to 100%.

Repeated insult patch tests on 206 patients with 4 hair dyes containing up to 2.144% p-Phenylenediamine found no irritation or sensitization. Hair dye composites containing up to 3.5% p-Phenylenediamine patch tested in another study involving 3500 subjects, for a total number of individual hair dye applications of 116,647, produced a total of 205 positive reactions in 163 female subjects; 8 reactions in 4 subjects were identified as allergic responses to the products.

A p-Phenylenediamine photopatch test was conducted on 1 subject; p-Phenylenediamine was not phototoxic. In a photosensitization study involving 23 patients, photocontact allergy to p-Phenylenediamine was observed in one patient and plain contact allergy was observed in a second patient.

In occupational studies involving hairdressers/barbers, p-Phenylenediamine sensitization rates in the range of 3.7% (2 of 54 tested) to 85% (28 of 33 tested) have been reported.

Positive patch test reactions to p-Phenylenediamine and p-Phenylenediamine HCl appear in numerous case reports, some of which also reported skin depigmentation at the p-Phenylenediamine patch test site. In cases in which skin depigmentation was observed at patch test sites, the patients evaluated either used hair dyes or various colorants (i.e., for the mustache, feet, or tattoos), and skin depigmentation was also noted in the areas of application of each hair dye/colorant.

Numerous case reports of dermatitis following henna tattoo application have also been identified in the published literature; positive (+ to +++) patch test reactions to p-Phenylenediamine were also observed. In one of the case reports, the skin paint for temporary tattoos contained 2.94% p-Phenylenediamine. Reportedly, p-Phenylenediamine is one of the additives that is used to accelerate drying and darken the reddish color of the henna.

Edema of the face, neck, ears, and scalp has occurred after hair dye use. Edema of the eyelids and conjunctiva and tearing have been observed and more severe reactions (damage to vision) have occurred after the application of p-Phenylenediamine-containing hair dyes to the eyebrows and eyelashes.

The most recent comprehensive review of available epidemiology studies concluded that there is insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. A summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml.

DISCUSSION

The Expert Panel recognizes that p-Phenylenediamine and its salts are used mostly in hair dyes and colors at concentrations of ≤6%, but that uses have been reported in tints and lighteners with color, with no available data on concentration. The Panel expects that use concentrations in these product categories will be no higher than for hair dyes and colors.

4-Aminobiphenyl, a known urinary bladder carcinogen, and 2-aminobiphenyl (carcinogenic, induces hemangiosarcomas) have been detected in batches of chemical research grade p-Phenylenediamine (purity of 97%). The Expert Panel states that cosmetic grade p-Phenylenediamine should not contain 4-aminobiphenyl or 2-aminobiphenyl, noting that the major U.S. manufacturer of p-Phenylenediamine produces this chemical (purity of > 99% p-Phenylenediamine) for use in

hair dyes via the process of direct nitration of benzene without chlorinating, which does not yield aminobiphenyl compounds, and not by the reduction of p-nitroaniline. Thus, it is the expectation of the Expert Panel that 99% pure p-Phenylenediamine (free of aminobiphenyls) is being used by the cosmetics industry.

p-Phenylenediamine may cause mutations, depending on the test system and test conditions. In the Ames test, different researchers used different solvents for the p-Phenylenediamine, different chemicals for induction, different metabolic activation systems, and slight modifications of the test procedure; any or all of these may explain the observed differences in results. The available studies do not suggest that p-Phenylenediamine is carcinogenic or teratogenic.

In considering hair dye epidemiology data, the CIR Expert Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other 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 application of hair dyes containing p-Phenylenediamine to the eyebrows and eyelashes can result in lost or permanently damaged vision. p-Phenylenediamine is a sensitizer for guinea pigs and for human beings. Phototoxicity and photosensitization data are limited, but suggest that sensitization is approximately the same with or without light. Hair dyes containing p-Phenylenediamine are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal Food, Drug and Cosmetic Act of 1938 when cautionary statements and patch test instructions are conspicuously displayed on the labels. While some persons may be sensitized under proper conditions of use, the Expert Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures.

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

The FDA has determined that uses of p-Phenylenediamine other than as a hair dye are unapproved. 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 — p-Phenylenediamine is a known sensitizer, highly inappropriate for such use 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 (for more information, see the FDA website at: http://www.cfsan.fda.gov/~dms/cos-tatt.html).

CONCLUSION

The CIR Expert Panel concluded that p-Phenylenediamine, p-Phenylenediamine HCl, and p-Phenylenediamine Sulfate are safe as hair dyes in the practices of use and concentration as described in this safety assessment.

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