Amended Safety Assessment of 2-Nitro-*p*-Phenylenediamine as Used in Cosmetics

Status:	Draft Final Amended Report for Panel Review
Release Date:	May 22, 2026
Panel Meeting:	June 15-16, 2026

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

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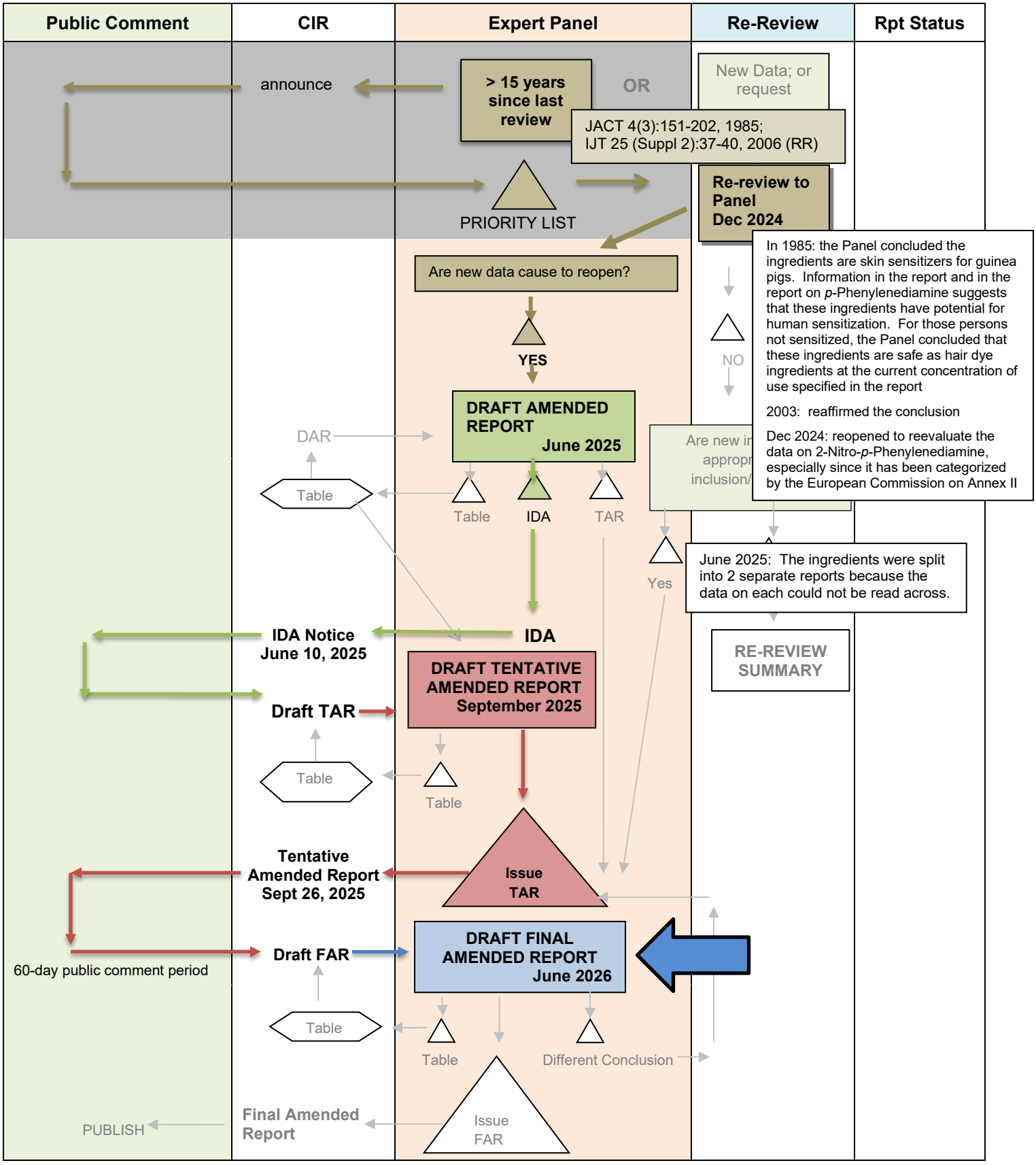
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY 2-Nitro-*p*-Phenylenediamine

MEETING June 2026



Cosmetic Ingredient Review

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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 22, 2026
Subject: Amended Safety Assessment of 2-Nitro-*p*-Phenylenediamine as Used in Cosmetics

Enclosed is the Draft Final Amended Report on the Safety Assessment of 2-Nitro-*p*-Phenylenediamine as Used in Cosmetics. (It is identified as *report_2-Nitro-p-Phenylenediamine_062026* in the pdf document.) At the September 2025 meeting, the Panel issued a Tentative Amended Report with a revised conclusion stating that the available data are insufficient to make a determination of safety for 2-Nitro-*p*-Phenylenediamine under the intended conditions of use as a hair dye ingredient. In order to come to a conclusion of safety for this ingredient, the following additional data are needed:

- maximum concentration of use in hair dye formulations
- a 90-d oral repeated dose study with a no-observed-adverse-effect level (NOAEL) that shows a dose-response relationship

Since the September meeting, CIR has updated the use data with RLD obtained from the FDA in 2025. 2-Nitro-*p*-Phenylenediamine is now reported to be used in 4 hair dyes and colors (1 additional use from that previously reported). No additional data have been received. Comments provided by the Council prior to the September 2025 meeting (*PCPCcomments1_2-Nitro-p-Phenylenediamine_062026* and *response-PCPCcomments1_2-Nitro-p-Phenylenediamine_062026*) and on the Tentative Amended Report (*PCPCcomments2_2-Nitro-p-Phenylenediamine_062026* and *response-PCPCcomments2_2-Nitro-p-Phenylenediamine_062026)* have been addressed.

Based on the equivocal relevance of certain genotoxicity study methods, CIR staff have excluded the following citations/studies from the report:

- a mutagenicity study using *S. cerevisiae* at up to 500 µg/ml - from original report
- sister chromatid exchange studies in Chinese hamster ovary cells (at up to 0.001M) and in hamsters (at up to 500 mg/kg, orally or up to 300 mg/kg intraperitoneally) – from first re-review
- an *E. coli* K-12 uvrB/recA DNA repair host-mediated assay in mice (up to 660 mg/kg, orally) in mice – from first re-review
- an immunological DNA synthesis-inhibition test (no further details available) – from first re-review

Additional supporting documents for this report package include a flow chart (*flow_2-Nitro-p-Phenylenediamine_062026*), the original report (*originalreport1985_2-Nitro-p-Phenylenediamine_062026*), the first re-review (*rereview2006_2-Nitro-p-Phenylenediamine_062026*), the data from the first re-review (*RRdata_2-Nitro-p-Phenylenediamine_062026*), report history (*history_2-Nitro-p-Phenylenediamine_062026*), a search strategy (*search_2-Nitro-p-Phenylenediamine_062026*), a data profile (*dataprofile_2-Nitro-p-Phenylenediamine_062026*), transcripts from the meeting at which the re-review was discussed (*transcripts_2-Nitro-p-Phenylenediamine_062026*), and the minutes from all the meetings at which 2-Nitro-*p*-Phenylenediamine was discussed during the original review (*originalminutes_2-Nitro-p-Phenylenediamine_062026*).

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

2-Nitro-*p*-Phenylenediamine History

1985– The CIR's Final Report on the Safety Assessment of 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine was published in the *JACT* after the report was finalized by the Panel in 1983. The Panel concluded:

> "[2-Nitro-*p*-Phenylenediamine] and [4-Nitro-*o*-Phenylenediamine] are skin sensitizers for guinea pigs. Information in this report and in the report on [*p*-Phenylenediamine] suggests that [2-Nitro-*p*-Phenylenediamine] and [4-Nitro-*o*-Phenylenediamine] have potential for human sensitization. For those persons not sensitized, the Expert Panel concludes that [2-Nitro-*p*-Phenylenediamine] and [4-Nitro-*o*-Phenylenediamine] are safe as hair dye ingredients at the current concentration of use."

November 2003 – Review of the available published literature since 1983 was conducted in accordance to CIR Procedures regarding re-review of ingredients after ~15 years. The Panel considered a re-review of this report and reaffirmed the 1985 conclusion.

2006 – The re-review summary of 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine was published in the *IJT*.

December 2024 – Review of the available published literature since 2003 was conducted in accordance to CIR Procedures regarding re-review of ingredients after ~15 years. The Panel re-opened the safety assessment for this ingredient, due to 2-Nitro-*p*-Phenylenediamine being banned for use in cosmetics by the European Commission.

June 2025 – The Panel split 4-Nitro-*o*-Phenylenediamine into a separate report from 2-Nitro-*p*-Phenylenediamine as the data from these 2 ingredients cannot be read across. The Panel issued an Insufficient Data Announcement for 2-Nitro-*p*-Phenylenediamine. The following information is required to determine the safety of this ingredient:

- Maximum concentration of use in hair dye formulations
- A 90-d oral repeated dose study with a no-observable-adverse-effect level (NOAEL) that shows a dose-response relationship
- Phototoxicity/photosensitization data

September 2025 – The Panel issued a Tentative Amended Report for public comment with the conclusion that the available data are insufficient to make a determination of safety for 2-Nitro-*p*-Phenylenediamine. The additional data needed to determine the safety of this ingredient are:

- Maximum concentration of use in hair dye formulations
- A 90-d oral repeated dose study with a no-observable-adverse-effect level (NOAEL) that shows a dose-response relationship

2-Nitro-*p*-Phenylenediamine Data Profile* – June 2026 – 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	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
2-Nitro-*p*-Phenylenediamine	XO	O	O	XO	XO	O	O	O		O	X O		O	O	XO	O		O		O			O				O	O

* "X" indicates that new data were available in a category for the ingredient. "O" indicates data were reported in the original safety assessment.

2-Nitro-*p*-Phenylenediamine

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
2-Nitro-*p*-Phenylenediamine	5307-14-2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Search Strategy (from 2003 forward)

PubMed
(2-nitro-p-phenylenediamine) OR (5307-14-2[EC/RN Number]) – 3 hits, 3 relevant

ECHA
An inactive dossier was found for 2-Nitro-*p*-Phenylenediamine.

SCCP/SCCS
No opinion found for 2-Nitro-*p*-Phenylenediamine.

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
- Connected Papers - https://www.connectedpapers.com/
- DeepDyve - https://www.deepdyve.com/

Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/winci/ingredient-custom-search/
- FDA Cosmetics page - https://www.fda.gov/cosmetics
- eCFR (Code of Federal Regulations) - https://www.ecfr.gov/
- FDA search databases: https://www.fda.gov/industry/fda-basics-industry/search-databases
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras
- SCOGS database: https://www.fda.gov/food/generally-recognized-safe-gras/gras-substances-scogs-database
- Inventory of Food Contact Substances Listed in 21 CFR: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=IndirectAdditives
- Drug Approvals and Database: https://www.fda.gov/drugs/development-approval-process-drugs/drug-approvals-and-databases
- FDA Orange Book: https://www.fda.gov/drugs/drug-approvals-and-databases/approved-drug-products-therapeutic-equivalence-evaluations-orange-book
- OTC Monographs - https://dps.fda.gov/omuf
- Inactive Ingredients Approved For Drugs: https://www.accessdata.fda.gov/scripts/cder/iig/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- 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/
- EUR-Lex - https://eur-lex.europa.eu/homepage.html
- Scientific Committees (SCCS, etc) opinions: https://health.ec.europa.eu/scientific-committees_en https://health.ec.europa.eu/scientific-committees/scientific-committee-consumer-safety-sccs_en
- ECHA (European Chemicals Agency – REACH dossiers) – https://echa.europa.eu/
- 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
- EFSA (European Food Safety Authority) - https://www.efsa.europa.eu/en
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- Office of Dietary Supplements https://ods.od.nih.gov/
- 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) IRIS library - https://apps.who.int/iris/
- a general Google and Google Scholar search should be performed for additional background information, to identify references that are available, and for other general information - www.google.com https://scholar.google.com/

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Kimberly Norman, Ph.D., DABT, ERT
Industry Liaison to the CIR Expert Panel

DATE: September 2, 2025

SUBJECT: Draft Tentative Amended Report: Amended Safety Assessment of 2-Nitro-p-Phenylenediamine as Used in Cosmetics (draft prepared for the September 8-9, 2025, meeting)

The Personal Care Products Council respectfully submits the following comments on the draft tentative amended report, Safety Assessment of 2-Nitro-p-Phenylenediamine as Used in Cosmetics.

Key Issues

There are several animal studies already summarized in the CIR report, including a 1979 NCI dietary exposure bioassay in rats and mice. This study also included a multidose 4-week dose-range finding study for which body weight and mortality are reported. Doses (mg/kg bw) would need to be calculated for these studies from dietary concentrations and a careful look at the data in the Appendices summarizing the non-neoplastic lesions would need to be completed.

An animal study should not be requested without a more careful look at the existing animal studies. Doses (mg/kg) from the dermal studies should also be calculated before they are dismissed for use as point of departures e.g., one 20-month study reported a NOAEL at 0.5 ml/kg for a formulation containing 0.85% 2-Nitro-p-Phenylenediamine applied 3x/week.

Rather than a lack of a 90-day animal study, the report should indicate that an MOE cannot be calculated because there is no information about current concentration of use of 2-Nitro-p-Phenylenediamine in hair dye products.

Additional Considerations

Definition and Structure; Dermal Penetration, old report summary – Please correct: “2-Nitro-p-Pheneylenediamine” (delete second “e”)

ADME, Parenteral, old report summary – Please correct “interperitoneally” to “intraperitoneally”

Developmental and Reproductive Toxicity, Parenteral, old report summary – Was a NOAEL identified in the subcutaneous study in mice treated on gestation days 6-15?

Carcinogenicity, old report summary – Please correct: “2-Nitro-p-Phenylenedimaine” (m and a are transposed)

Retrospective and Multicenter Studies, old report summary – As 2-Nitro-p-Phenylenediamine is the ingredient of concern, it should not say that “Cross reactions were not observed” with “and/or 2-Nitro-p-Phenylenediamine”.

Case Reports – Please correct: “methylenediamiline” to “methylenedianiline”

Risk Assessment – As noted above, rather than a lack of a point of departure, please state that exposure cannot be calculated because of a lack of a use concentration.

2-Nitro-*p*-Phenylenediamine – June 2026 – Christina Burnett	
Comment Submitter: Kimberly Norman, Ph.D., Personal Care Products Council **Date of Submission: September 2, 2025**	
Comment	**Response/Action**
Key Issue: There are several animal studies already summarized in the CIR report, including a 1979 NCI dietary exposure bioassay in rats and mice. This study also included a multidose 4-week dose-range finding study for which body weight and mortality are reported. Doses (mg/kg bw) would need to be calculated for these studies from dietary concentrations and a careful look at the data in the Appendices summarizing the non-neoplastic lesions would need to be completed. An animal study should not be requested without a more careful look at the existing animal studies. Doses (mg/kg) from the dermal studies should also be calculated before they are dismissed for use as point of departures e.g., one 20-month study reported a NOAEL at 0.5 ml/kg for a formulation containing 0.85% 2-Nitro-p-Phenylenediamine applied 3x/week. Rather than a lack of a 90-day animal study, the report should indicate that an MOE cannot be calculated because there is no information about current concentration of use of 2-Nitro-p-Phenylenediamine in hair dye products.	Risk Assessment section was removed per the direction of the Panel at the September 2025 meeting. The Panel upheld the need for a 90-d repeated dose study during their deliberations at the meeting.
Definition and Structure; Dermal Penetration, old report summary – Please correct: "2-Nitro-p-Pheneylenediamine" (delete second "e")	Corrected.
ADME, Parenteral, old report summary – Please correct "interperitoneally" to "intraperitoneally"	Corrected.
Developmental and Reproductive Toxicity, Parenteral, old report summary – Was a NOAEL identified in the subcutaneous study in mice treated on gestation days 6-15?	Paragraph was revised to add "the highest 'no effect' dose of 2-Nitro-p-Phenylenediamine was 64 mg/kg/d.
Carcinogenicity, old report summary – Please correct: "2-Nitro-p-Phenylenedimaine" (m and a are transposed)	Corrected.
Retrospective and Multicenter Studies, old report summary – As 2-Nitro-p-Phenylenediamine is the ingredient of concern, it should not say that "Cross reactions were not observed" with "and/or 2-Nitro-p-Phenylenediamine".	Corrected.
Case Reports – Please correct: "methylenediamiline" to "methylenedianiline."	Corrected.
Risk Assessment – As noted above, rather than a lack of a point of departure, please state that exposure cannot be calculated because of a lack of a use concentration.	Risk Assessment section was removed per the direction of the Panel at the September 2025 meeting.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Kimberly Norman, Ph.D., DABT, ERT
Industry Liaison to the CIR Expert Panel

DATE: October 9, 2025

SUBJECT: Tentative Amended Report: Amended Safety Assessment of 2-Nitro-p-Phenylenediamine as Used in Cosmetics (release date: September 26, 2025)

The Personal Care Products Council respectfully submits the following comments on the tentative amended report, Safety Assessment of 2-Nitro-p-Phenylenediamine as Used in Cosmetics.

Key Issue

Studies of hair dyes containing 2-Nitro-p-Phenylenediamine from the original report often indicated that the dye was diluted (usually 1:1) with a hydrogen peroxide solution before application. In the current draft, only the original concentration of 2-Nitro-p-Phenylenediamine is stated, and the fact that it was diluted before application is not stated. The applied concentration and volume applied (if available) should be stated for each hair dye study so that an approximate application dose could be calculated.

Additional Considerations

Introduction – Please correct: “in the original [report] could not be read across” (add “report”)

Non-Cosmetic Use – Please add the uses indicated in the ECHA dossier (agrochemical intermediate, chemical synthesis, pharma intermediate, dyestuff application, laboratory chemicals).

ADME, Animal, Oral, old report summary – In the rat study, did they really confirm that the parent compound was excreted in the urine and feces, or did they just measure the excretion of radioactivity?

Short-Term, old report summary – Please check the original report again. When available, doses in terms of mg (or g)/kg bw should be presented. The description of the dermal 20-day study in rabbits says “and 0.13”. This needs to be deleted as the 0.013% concentration refers to the concentration of 4-Nitro-o-Phenylenediamine.

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

The summary in the new report should indicate that the hair dye was diluted and state the mg/kg bw doses that were used.

Subchronic, Dermal, old report summary – Please make it clear that both the 0.55% and 1.1% hair dyes were diluted 1:1 with a hydrogen peroxide solution before testing. The volume of material applied should be stated. It is also not clear that the hair dye containing 0.55% was tested once every week and the hair dye containing 1.1% was tested twice a week.

Developmental and Reproductive Toxicity, Dermal, old report summary – Please review the original report as there are many inconsistencies between the original report and the summary in this report in this section. For example, the 3-generation study was completed in rats, not mice (and only one dye containing 2-Nitro-p-Phenylenediamine was studied). The hair dye was mixed with hydrogen peroxide before application.

The original report describes a rabbit developmental toxicity study which is not included in the new report. "The same hair dye formulation (1.1 percent 2NPPD and 0.25 percent 4NOPD) was applied topically in a dose of 2 ml/kg to the clipped backs of more than 30 female rabbits two times a week for 4 weeks prior to mating and throughout mating and gestation. Thirty of the rabbits were mated, 21 became pregnant, and 4 of those mated died. (Thirty-two untreated control rabbits were mated, 21 became pregnant, and 6 of those mated died.) No signs of maternal toxicity were observed. There were no adverse effects on pregnancy rates and maternal survival and body weights. Focal alopecia was noted at slightly higher incidences in treated rabbits during the first two thirds of gestation; in the last third of gestation, the incidence of alopecia in control and treated rabbits was similar. The mean numbers of corpora lutea,

implantations, and live fetuses, implantation efficiency, and number of doses with two or more resorptions were comparable in control and treated rabbits. There was no evidence of a teratogenic effect. Embryotoxicity may have occurred, as the percent of live fetuses was significantly less in the treated rabbits (85.9 percent in the treated rabbits and 93.8 percent in the control rabbits), and the percent of resorbed fetuses was significantly greater (14.1 percent in the treated rabbits and 6.2 percent in the control rabbits)."

Although the summary mentions a 2-generation study in rats of the hair dye containing 1.1% 2-Nitro-p-Phenylenediamine, this is probably the 3-generation study for which the summary incorrectly says was done in mice.

Carcinogenicity – Whenever possible, mg/kg bw doses should be stated. The ECHA dosser states that the NOAEL for the NCI 78-week rat study was 110 mg/kg/day.

2-Nitro-*p*-Phenylenediamine – June 2026 – Christina Burnett	
Comment Submitter: Kimberly Norman, Ph.D., Personal Care Products Council **Date of Submission: October 9, 2025**	
Comment	**Response/Action**
Key Issue: Studies of hair dyes containing 2-Nitro-p-Phenylenediamine from the original report often indicated that the dye was diluted (usually 1:1) with a hydrogen peroxide solution before application. In the current draft, only the original concentration of 2-Nitro-p-Phenylenediamine is stated, and the fact that it was diluted before application is not stated. The applied concentration and volume applied (if available) should be stated for each hair dye study so that an approximate application dose could be calculated.	Summarizes were revised to include dilution and application details.
Introduction – Please correct: "in the original [report] could not be read across" (add "report")	Corrected.
Non-Cosmetic Use – Please add the uses indicated in the ECHA dossier (agrochemical intermediate, chemical synthesis, pharma intermediate, dyestuff application, laboratory chemicals).	Added.
ADME, Animal, Oral, old report summary – In the rat study, did they really confirm that the parent compound was excreted in the urine and feces, or did they just measure the excretion of radioactivity?	Corrected to state radioactivity was absorbed.
Short-Term, old report summary – Please check the original report again. When available, doses in terms of mg (or g)/kg bw should be presented. The description of the dermal 20-day study in rabbits says "and 0.13". This needs to be deleted as the 0.013% concentration refers to the concentration of 4-Nitro-o-Phenylenediamine. The original report includes this description of a rabbit study: "A hair dye composite containing 0.55 percent 2NPPD was diluted 1:1 with a hydrogen peroxide activator and applied in doses of 1.0, 2.0, and 4.0 g/kg per day for 20 days to the shaved backs of groups of male and female rabbits (number unspecified). The application site was approximately 10 percent of the body surface. There was also a group of untreated control animals. The skin of 2 animals from each group was abraded prior to composite application. The rabbits were observed for a further 14 days after the test period. Two rabbits died during the test period in the 1.0 g/kg group, 2 died in the 2.0 g/kg group, 1 died in the 4.0 g/kg dye-treated group, and 1 died in the control group. These deaths were attributed to naturally occurring disease; the incidence and severity of disease may have been increased due to the stress of the severe local skin reactions and the dosing procedure. There were adverse effects on body weight in the treated rabbits during the test period, but body weights were comparable to the controls during the observation period. There were no significant adverse findings in the hematological and clinical chemistry parameters or in the urinalyses. No clinical signs of toxicity were observed. From Day 5 to Day 20 of the test period, local skin reactions were characterized by escharosis, with subsequent sloughing of the skin at the application site in the treated animals. By the end of the 14day observation period, the skin appeared normal. No significant gross or microscopic alterations were observed in the tissues and organs of the rabbits killed at the end of the study or in any treated animals that died during the study, except for the skin. At the dye application site in a few animals, edema and/or hyperkeratosis was observed." The summary in the new report should indicate that the hair dye was diluted and state the mg/kg bw doses that were used.	Doses added and the deletion of the irrelevant concentration was made. Information on dilution and doses was added.
Subchronic, Dermal, old report summary – Please make it clear that both the 0.55% and 1.1% hair dyes were diluted 1:1 with a hydrogen peroxide solution before testing. The volume of material applied should be stated. It is also not clear that the hair dye containing 0.55% was tested once every week and the hair dye containing 1.1% was tested twice a week.	Summary revised with more details.

2-Nitro-*p*-Phenylenediamine – June 2026 – Christina Burnett	
Comment Submitter: Kimberly Norman, Ph.D., Personal Care Products Council **Date of Submission: October 9, 2025**	
Comment	**Response/Action**
Developmental and Reproductive Toxicity, Dermal, old report summary – Please review the original report as there are many inconsistencies between the original report and the summary in this report in this section. For example, the 3-generation study was completed in rats, not mice (and only one dye containing 2-Nitro-p-Phenylenediamine was studied). The hair dye was mixed with hydrogen peroxide before application. The original report describes a rabbit developmental toxicity study which is not included in the new report. "The same hair dye formulation (1.1 percent 2NPPD and 0.25 percent 4NOPD) was applied topically in a dose of 2 ml/kg to the clipped backs of more than 30 female rabbits two times a week for 4 weeks prior to mating and throughout mating and gestation. Thirty of the rabbits were mated, 21 became pregnant, and 4 of those mated died. (Thirty-two untreated control rabbits were mated, 21 became pregnant, and 6 of those mated died.) No signs of maternal toxicity were observed. There were no adverse effects on pregnancy rates and maternal survival and body weights. Focal alopecia was noted at slightly higher incidences in treated rabbits during the first two thirds of gestation; in the last third of gestation, the incidence of alopecia in control and treated rabbits was similar. The mean numbers of corpora lutea, implantations, and live fetuses, implantation efficiency, and number of doses with two or more resorptions were comparable in control and treated rabbits. There was no evidence of a teratogenic effect. Embryotoxicity may have occurred, as the percent of live fetuses was significantly less in the treated rabbits (85.9 percent in the treated rabbits and 93.8 percent in the control rabbits), and the percent of resorbed fetuses was significantly greater (14.1 percent in the treated rabbits and 6.2 percent in the control rabbits)." Although the summary mentions a 2-generation study in rats of the hair dye containing 1.1% 2-Nitro-p-Phenylenediamine, this is probably the 3-generation study for which the summary incorrectly says was done in mice.	Old report summary on dermal DART was revised.
Carcinogenicity – Whenever possible, mg/kg bw doses should be stated. The ECHA dosser states that the NOAEL for the NCI 78-week rat study was 110 mg/kg/day.	The data for the study from the National Cancer Institute were summarized from the original report and the dose values were reported in ppm, which was how the original source also reported the values. The NCI report did not include an NOAEL. ECHA, which is not always reliable in summarizing or reporting data, appears to have extrapolated the data for the mg/kg bw doses and NOAEL.

DECEMBER 2024 PANEL MEETING – RE-REVIEW

Belsito Team – December 2, 2024

DR. BELSITO: Oh, no, I'm sorry. 2-nitro-paraphenylenediamine. I wanted to get rid of these hair dyes, didn't I?

MS. BURNETT: That's all I have this time.

DR. BELSITO: Okay, so again we have Wave 2 PCPC comments that I agreed with. Panel?

MS. BURNETT: I did, I'm sure.

DR. SNYDER: Agreed.

DR. BELSITO: Okay.

MS. BURNETT: Hold on, we're on nitro-phenylenediamine?

DR. BELSITO: Yeah.

DR. RETTIE: So, this is a re-review?

MS. BURNETT: Yes.

DR. BELSITO: Yeah, so this is a re-review of 2-nitro-paraphenylenediamine and 4-nitro-paraphenylenediamine from 1985. We concluded they were -- they had this long thing about skin sensitizers but essentially safe as a hair dye ingredient current concentration of use. We reviewed it in 2003 -- reaffirmed the '85 conclusion and it was published in 2006. So, it's been 15 years since the re-review, and we need to consider whether the safety assessment needs to be reopened so that's where we are.

So basically my thought was that we have 2-nitro and 4-nitro-paraphenylenediamine here and really our entire safety of the 2-nitro is based upon data from the 4-nitro and I think -- again, this is where I would like Dr. Sam Cohen's thoughts about carcinogenicity on the read-across but I just would have concerns about reading across from the 4 to the 2 and I think the E.U. may have gotten it right with differentiating between the two of these and just was thinking that perhaps we should reopen it to consider our safety on the 2-nitro which is banned by the E.U. Is that correct, Christina?

MS. BURNETT: Yes.

DR. BELSITO: And I'm assuming these other hair uses would include eyelashes and eyebrows, is that correct in our RLD? Other hair coloring preparations, there were 11.

MS. BURNETT: Oh. So, it's the other preparations category at the very bottom is literally a category of, we don't know what it's being used as. It has not been categorized in any hair dyes, eye products, anything like that. It's just the final catch-all of uses. So, we do not know.

DR. BELSITO: So, it could include eyelash and eyebrow?

MS. BURNETT: It could. We have no idea what it is.

DR. BELSITO: But my biggest concern here was reading from the 4 to the 2 because we have absolutely zero data on the 2. So, I will leave that to other people what your thoughts are. Curt, Paul, Allan?

DR. SNYDER: Yeah. It's beyond my expertise the read across from a 2 to a 4 or a 4 to a 2 so I support reopening and to pursue it.

DR. RETTIE: Yeah, I mean the nitro groups -- this is just paraphenylenediamine with a couple of nitro groups on them. The nitro groups are deactivating so make it less reactive but -- I support.

DR. BELSITO: Less reactive for all tox endpoints, Allan?

DR. RETTIE: Well, no. No, no. Not all tox endpoints, just the oxidative activation of the amino portion of it. I'd support reopening because of the high use of the 4 and there's also an increased maximum concentration now of 0.33. We have some new studies, some of which add new information. So, for sure, I think we reopen it. It'd be nice to hear what Dr. Cohen says about this when he joins us.

DR. BELSITO: Curt?

DR. KLAASSEN: Yeah, I agree. I think we should reopen.

DR. BELSITO: Okay.

DR. EISENMANN: My question is will you continue to review this in the same report, or will you do two separate reports? Because if you can't read across should they be on the same report? That's the question.

DR. BELSITO: Well, I mean, I think maybe this should go to the Read-Across Working Group, number one. Number two, perhaps we could ask Dr. Samuel Cohen to do a little pre-March recommendation looking at this from the carcinogenicity endpoint whether he would be comfortable reading across the carcinogenicity data from 4-nitro-PPD to a 2-nitro.

DR. SNYDER: Well, I think at a minimum the title has to change because the title's currently 2-nitro.

MS. BURNETT: It's 2-nitro-p-phenylenediamine and 4-nitro-o-phenylenediamine.

DR. SNYDER: Oh, okay, I'm sorry. Never mind. You're right. I'm sorry, you're right. I think we have a path forward, Don.

DR. BELSITO: Okay, so we're going to recommend reopening to determine whether and recommend that it go -- before we actually look at it again -- go to the Read-Across Working Group to determine whether we can use the data 4-nitro-o-phenylenediamine to satisfy data needs for the 2-nitro-para and perhaps ask Dr. Cohen to give us an off the cuff recommendation as to whether the carcinogenicity data can be read across and if the one or both of those responses are that we're having trouble with the read-across then we would need two separate reports.

DR. RETTIE: Yeah, the read-across committee is -- so far has come down pretty much unanimously in favor of not reading across for highly sensitive endpoints like carcinogenicity so I suspect that's where we would be. But, yeah, I'm definitely interested in hearing Dr. Cohen's opinion.

DR. BELSITO: Okay. Yeah, I mean, I think that the Europeans may have gotten this right. So, we're going to reopen for question of read-across and especially in carcinogenicity. Okay. Anything else?

Cohen Team – December 2, 2024

DR. COHEN: 2-nitro-paraphenylenediamine and 4-nitro-*o*-phenylenediamine was originally published in 1985 with a conclusion as safe as a hair dye ingredient in the current concentration. The Panel considered a re-review report in 2003 but reaffirmed the 1985 decision and published it in 2006. It's been 15 years. The FDA RLD has its use in three hair coloring preparations. The VCRP had one use in other non-coloring hair preparations. A hair color rinse and a hair color shampoo. Does that make sense? Oh, in each of those. And the Council's maximum concentration was up to one percent for 2-nitro-paraphenylenediamine.

For 4-nitro-*o*-phenylenediamine there were 143 uses from the RLD with maximum concentration of use up to 0.2 percent. In 2022 -- I'm sorry. I'm sorry, let me correct. In 2003 it was 0.2 percent. In 2022 the max concentration for 4-nitro-*o*-phenylenediamine was 0.33 in hair dyes and colors. There's new data that we see. There's an SCCP MOS of 357. But there's new data there and I'd like to call it out for a discussion for do we open?

DR. TILTON: So, in this case, we do have quite a bit of new data for 4-nitro, dermal penetration, toxicokinetics, repeat dose toxicity, genotoxicity, sensitization. I noted that many of the results are consistent with prior studies, but we are seeing continued reports of use of 2-nitro and an increase in concentration in the 4-nitro. And I guess for those reasons I had considered reopening.

DR. COHEN: I had that too but I --

DR. BERGFELD: I had that too.

DR. COHEN: Did you conclude that? There was just so much in there and I know that we have the option for an expanded re-review report.

DR. BERGFELD: I could go with that.

DR. ROSS: Yeah, I had that flagged as a possibility. When I looked at this, yeah, I mean, there was a lot of new data, and it would give us the opportunity to include MoE calculations into consideration of safety. But I thought it was relatively minor changes in concentration. The SCCS MoE was up to 0.5 percent on head and our concentration saw an increase up to 0.33 percent for 4-nitro, so we're still in that band.

So, the conclusion probably wouldn't be any different and so I thought that fit the criteria for an expanded re-review and so that's where I was coming down on this one. But I'm happy to go with the flow. I think we could probably do an expanded re-review.

DR. COHEN: We actually don't know what that's going to look like. We have no idea what that really -- I mean, does it look like this? Is it basically this with all the tables?

MS. FIUME: So, it would be the re-review summary that you would normally see but all of the tables would be included with it.

DR. COHEN: Yeah, I mean, isn't this sort of made to order for that new thing because what got me was all the new data, right, and it was before I read the administrative memo, I was reviewing it before that. And then afterwards I kind of came

around with like am I going to change the final conclusion and the answer's probably not. But Susan, what do you think of that? I mean, we came to the same conclusion, but I hemmed and hawed on it.

DR. TILTON: Yeah, I mean, I think now that the extended re-review is an option and we can include this data -- I mean, like I said, I noted that even though there was a lot of new data, many of the results were consistent with the prior studies so I think that could be summarized in the extended re-review summary.

DR. COHEN: Right. I mean, there's a couple of comments where it says the original report did not have robust ADME data but is any of that data changing our conclusion?

DR. BERGFELD: No.

DR. COHEN: Okay. So, we can go out with an expanded re-review. Do not reopen.

DR. ROSS: That would be my preference. You're presenting this one, David, right?

DR. COHEN: I am, indeed.

DR. ROSS: Yeah.

DR. BERGFELD: I think it's a great way to start to introduce this new format.

DR. ROSS: Yeah. Let me just look at this absorption data again. I didn't flag it the first time.

DR. COHEN: It's not in the original report.

DR. ROSS: Yeah. I didn't flag anything about it the first time but I'm not seeing anything that would really change our conclusions. But since you do have that you can put an MoE value in, but I don't think it would change your conclusions, so I think our discussion stands.

DR. COHEN: Okay. Bart, any comments on it? Maybe we'll try for this. It'll still be interesting to see what the format looks like and how it publishes. So, when we publish it, it goes out as this re-review summary, does it hotlink to the original report?

DR. HELDRETH: It does not do any special linking to the report, it's just its own article in the issue of IJT and it references the original article that you scroll down to the bibliography and it's going to have the original article -- re-review -- posted there as one and this conclusion supersedes that. Now, it's unchanged, but the --

DR. COHEN: Okay.

DR. ROSS: So, you see any problems with this, Bart, as an expanded re-review? I mean, obviously it depends on the discussion tomorrow.

DR. HELDRETH: I don't see a problem with it, per se, the only thing that I'm sitting here thinking about is the conclusion is very specific to hair dye, right? And if we look at the RLD for the 4-nitro-orthophenylenediamine, I guess those things fit under hair dye. I mean, hair dyes and colors, hair tint. Other hair coloring preparations. Other preparations. I guess it all fits under there I just think we have to be ready to say, yeah, that still fits with hair dye.

DR. COHEN: Well, we're only approving it as a hair dye. They reported this as a whole other thing and use it as a whole other thing but there's nothing in a reopened report that's going to change the conclusion other than it being approved as a hair dye. That's not changing.

DR. HELDRETH: Agree. Agree.

DR. COHEN: So, there are times as we discuss for PPD where the RLD starts to drive a little of the conversation. I'm not sure it should, but it just does sometimes.

DR. BERGFELD: Well, that's going to fall out over time. We'll figure out how to deal with that -- those high numbers.

DR. HELDRETH: Right. Once we have a baseline then it won't seem so high.

DR. COHEN: Christina, is there anything else we need right now?

MS. BURNETT: Not for this one, no. We're good.

Full Panel – December 3, 2024

DR. COHEN: We have both 2-Nitro-p-Phenylenediamine and 4-Nitro-o-Phenylenediamine, which was published in 1985 with the conclusion that these chemicals are skin sensitizers for Guinea pigs and that they have the potential for human sensitization. For those persons not sensitized, the Panel concluded that they were safe as hair dye ingredient in the current concentration of use.

The Panel previously considered a re-review in 2003 and reaffirm the original conclusion of 2006. It's been 15 years at least since then.

The RLD received information on 2-Nitro-p in three hair coloring products and the VCRP had it in three items. We have for the 4-Nitro-o, 143 products. I'm sorry for the 2-Nitro-p it was up to 1 percent. And for the 4-Nitro-o the maximum use went from .2 percent in 2003 to .33 percent in a 2022 survey. There is substantial new data. Of note, the EU has the 2-Nitro-p in Annex 2 and the 4-Nitro-o in Annex 3.

We thought that this may be an opportunity, because of the volume of data, to do an expanded re-review without reopening.

DR. BELSITO: I disagree.

DR. COHEN: You want to reopen?

DR. BELSITO: Yeah. We have zero data on the 2-Nitro. We were hit before with the ability to read across with these various differences in the structure on the ring. And I would really like your namesake, when he comes on board in March, to discuss whether the carcinogenicity data is transferable from the 4 to the 2-Nitro. We have zero data on 2-Nitro and I'm just not sure that we can read across from 4 to 2.

DR. COHEN: Team are we okay with that? We went back and forth a bit on this. David, Susan?

DR. ROSS: Yeah, I'm okay if you want to reopen it.

DR. TILTON: Yes.

DR. ROSS: I mean, we were plus/minus on this reopening and then we discussed the expanded re-review. I think the basis for Don's summary there was a good one.

DR. COHEN: Yeah. I think it's compelling. Susan?

DR. TILTON: Yes, I agree.

DR. RETTIE: Given our recent experience with the hair dye isomers, do we need to consider splitting out the 2-Nitro from the 4-Nitro?

DR. BELSITO: I think we need to get your input in terms of read across and Dr. Sam Cohen's input in terms of read across for genotoxicity before -- I mean, we just need more data.

DR. RETTIE: Yeah. Okay.

DR. BERGFELD: David, do you want to restate your motion?

DR. COHEN: Our motion is to reopen.

DR. BERGFELD: Is there a second? Don, are you seconding?

DR. BELSITO: Second. Yeah.

DR. BERGFELD: Any other comments? I'm going to call the question. Those opposing? Abstaining? It's approved. This will be reopened.

JUNE 2025 PANEL MEETING – DRAFT AMENDED REPORT

Belsito Team – June 9, 2025

DR. SNYDER: All right. The next one is 2-Nitro-p-Phenylenediamine. And so, this is a Draft Amended Report. In 1985, we published that there was potential for sensitization. For those not sensitized, it was safe as used. In 2003, we reaffirmed that conclusion.

In December of 2024, we reopened due to the European Commission on Annex II, putting it on their list of substances prohibited in cosmetics. We've received no new data for 2-Nitro-p-Phenylenediamine.

For 2, currently it's in 3 hair dye coloring preps from 0.1 to 1 percent. 4-Nitro is in 3 uses; originally, it was in 0.1 to 0.2 percent in 2023. Those concentration of uses have decreased in 2025, on the data, to 0.05 and 0.075 for maximum concentration of use.

So if no further data are needed, we should go with a Tentative Amended Report. If we need further data, then we want to go with the Insufficient Data Announcement. So what was the group's opinion about this?

DR. RETTIE: I just had a question about grouping them together. When we've looked at aromatic amine dyes, intensively over the last 18 months, we've split them all out so that they're in single reports. And this is one with two amines in the same report.

Have we given consideration to splitting them out? Of course, if the conclusions are the same then maybe that’s just busy work. But I was just struck that we were adopting a different approach with this one.

DR. BELSITO: I had the same question, Allan. Why are we grouping them? You know, again, we had this issue that you couldn't read across with the others. I mean, you're the read-across expert. Do you feel you can read across from one to the other for tox endpoints?

DR. RETTIE: Nope.

DR. SNYDER: Okay.

MS. BURNETT: This was a reopened report, so the original report had them grouped together. And we were waiting to see if you wanted to split them or keep them together.

DR. SNYDER: Well, it sounds like Allan's preference is that we suggest that we split them into two separate reports.

DR. BELSITO: That was my preference as well.

DR. KLAASSEN: Me too.

MS. BURNETT: Okay.

DR. RETTIE: That way we're consistent with what we've been doing the last year and a half.

DR. SNYDER: Okay. All right.

DR. BELSITO: Right. I think we have enough data on both of them, but it doesn't make sense to keep them together.

DR. SNYDER: Then they both go safe as used, correct?

DR. BELSITO: Well, we have no reported use concentration for the 2-Nitro-p-Phenylenediamine, right?

DR. SNYDER: Well, I thought we had -- in my notes, it's currently in three hair dye coloring preps from 0.1 to 1 percent. Is that not correct?

DR. RETTIE: For the 2-Nitro?

DR. SNYDER: Yeah, that's what I thought I had.

MS. BURNETT: No concentration reported in the most recent survey for 2-Nitro. Previously, in the 2003 report, it was used up to 1 percent.

DR. SNYDER: Okay.

MS. BURNETT: Yeah, so there's no current.

DR. RETTIE: We have .075 for the 4-Nitro, but nothing for the 2-Nitro.

DR. SNYDER: Okay.

DR. BELSITO: Right.

DR. SNYDER: So that one go insufficient for concentration of use.

DR. BELSITO: And I think that the 2-Nitro was probably prohibited in Europe because of that. But, I mean, it also penetrates pretty quickly. So, I thought the 4-Nitro PPD was safe as used in hair dyes. The 2-Nitro PPD, at this point, I thought was insufficient for concentration of use.

If we're going to split them and say where we're going with these, that's what I had.

DR. SNYDER: I agree. Everybody agree here? Allan?

DR. RETTIE: I'm good. Anyone want to push it for phototox for 2?

MS. BURNETT: If you're putting out an IDA, you can request what you want.

DR. RETTIE: Just trying to be consistent with what we've done in the last six months. It seemed to me that we didn't have any phototox data.

DR. SNYDER: Do you also want to put phototox on here?

DR. RETTIE: But it's a hair dye, so, I'd be guided by Panel members. I don't have a pressing concern.

DR. SNYDER: You think we need phototox also for the 2-Nitro, Don, even though -- I mean, it's a hair dye.

DR. BELSITO: I mean, it would be hard to imagine someone getting their hair dyed out in the sun. And, I mean, these are permanent hair dyes. Once they are absorbed into the hair, there's nothing that's really released on the skin. I mean, the problem is what gets on the skin during the hair dye. But I can't imagine someone leaving a salon with hair dye still on their skin.

DR. SNYDER: I agree.

DR. RETTIE: Yeah.

DR. SNYDER: We'll just leave it out.

DR. RETTIE: Yes.

DR. KLAASSEN: But that which is absorbed into the bloodstream, while small, it could be happening. And, you know, we do have data that says 30 percent is absorbed by monkeys and 17 percent in pigs within 24 hours.

DR. BELSITO: This is for the 2-Nitro, right, Curt?

DR. KLAASSEN: Yeah. Well, I don't know, I didn't note which one it was. I guess I would ask for it. I don't think it's a huge problem, but.

DR. RETTIE: I mean, 18 to 30 percent of radiolabel from these species, again, just seemed high to me.

DR. KLAASSEN: Yes.

DR. RETTIE: But, you know, that's the data we have. We seem to be confronted with some data that perhaps we would look askance at, but it is what we have in front of us, so we have to deal with that.

DR. KLAASSEN: They are small molecules.

DR. SNYDER: So what's the choice preference? Do you want to ask for the phototox?

DR. BELSITO: I mean, we can certainly ask for it. But, yeah, I mean, the study -- the details, it's on PDF Page 18, Curt, that you're referring to. So, it was measured in humans and monkey skin. Rapid penetration of the material was absorbed. We don't really have any further data on that.

The next one is on EPISKIN reconstructed skin model. And it looks like it's just not being mixed with peroxide. So it's the pure chemical just being put on the skin, which is not how it would be used as a hair dye. And I suspect the older studies from the old report were similar. But we don't have details, it's just a summary.

DR. SNYDER: We can just ask for clarification, then.

DR. BELSITO: I'm having a hard time hearing you, Paul.

MS. BURNETT: You have to put the mic on.

DR. SNYDER: I'm sorry, that's off. Sorry, Don. I said we could just raise that, since it's going out IDA, and then clarification as to the absorption in formulation.

DR. BELSITO: Right.

DR. SNYDER: Yeah.

DR. BELSITO: So the 2-Nitro, then, we need concentration of use in dermal absorption under conditions of use? Is that how you would state it?

DR. SNYDER: Or phototox data, but I guess I could change it to --

DR. BELSITO: And phototox data, sure.

DR. SNYDER: Okay.

DR. BELSITO: Then do we want phototox data on the 4-Nitro?

DR. RETTIE: Didn't we have it? I didn't specifically note that we needed that.

DR. BELSITO: I mean, has anyone looked for an absorption spectrum on these?

DR. RETTIE: They're bound to absorb where it would be problematic, just because they are aromatic amines. But there's phototox data on the 4 in Table 5. That's what I was referring to.

DR. BELSITO: Okay.

DR. RETTIE: But only that, no 2.

DR. SNYDER: Okay. So, I got three data needs for 2-Nitro: concentration of use, skin absorption as in formulation, and phototox.

DR. BELSITO: Yeah.

DR. SNYDER: And the other one is safe as used, the 4-Nitro.

DR. BELSITO: Yeah.

DR. SNYDER: Okay.

DR. RETTIE: Well, 4-Nitro was not phototoxic in the one entry there.

DR. SNYDER: Okay.

DR. RETTIE: Or the two entries, so.

DR. BELSITO: Yeah. So for the 4-Nitro in the Discussion, there's low dermal absorption under concentration of use as opposed to the 2. There's mixed genotox, but the carcinogenicity is negative. There's sensitization, but there's a coal tar exemption. That's what I had for the Discussion.

DR. SNYDER: Okay.

DR. RETTIE: So where did we land on the phototoxicity given that the 4-Nitro was negative? Are we still asking for it?

DR. SNYDER: For the 2.

DR. BELSITO: We're asking for it.

DR. RETTIE: Okay.

DR. SNYDER: For the 2, not the 4. I've got safe as used in hair dyes for the 4; and Discussion having a low dermal absorption compared to as with 2-Nitro. For 2-Nitro, we've got insufficient for concentration of use, skin absorption as used in formulation, and phototox.

DR. BELSITO: Yeah.

DR. SNYDER: Good.

MS. BURNETT: And we need to have two separate reports.

DR. SNYDER: And two separate reports.

DR. BELSITO: Yeah.

DR. SNYDER: Perfect.

Cohen Team – June 9, 2025

DR. DAVID COHEN: Okay, so 2-Nitro-p-Phenylenediamine and 4-Nitro-o-Phenylenediamine. We published a review on the safety of these two in 1985, with the conclusion that both are skin sensitizers for guinea pigs. Information in this report and in the report on these two have potential for human sensitization. For those persons not sensitized, the expert Panel concludes that the two are safe as hair dye ingredients at the current concentration of use. That is a very complicated conclusion.

The Panel previously considered re-review of the report in 2003, and reaffirmed the 1985 Conclusion as published in 2006. At the December 2024 meeting, the Panel reopened the safety assessment for these ingredients to reevaluate the data on 2-Nitro, especially since it's been categorized by the EC on Annex II, the list of substances prohibited in cosmetic use.

With no published opinion, categorization was presumably due to no reported use in the EU. While these Nitrophenylene Diamine ingredients are positional isomers, structural activity relationships between chemicals with different substitution patterns around an aromatic ring are equivocal at best; thereby, the use of read across from one ingredient to the other was challenging.

Since the December meeting, the only new data received has been an updated concentration of use survey by the Council. According to FDA RLD, in 2024, 2-Nitro-p-Phenylenediamine is reported to be used in three hair coloring preparations.

In 2002, notably higher uses were reported in the VCRP. 2-Nitro-p-Phenylenediamine was noted in 113 cosmetic formulations, and all uses reported in hair dye and colors. No concentration of use was reported in the Council's 2025 survey, whereas the Council's 2003 survey had a concentration up to 1 percent.

For 4-Nitro-o-Phenylenediamine, the RLD reported 143 uses in hair dye. And the VCRP had three uses in hair dye. Max concentration in 2003 was 0.2 percent. In 2025, 4-Nitro was used at up to 0.075 percent in hair dyes and colors.

Okay, so with the read-across issue, what is our opinion about this?

DR. SAM COHEN: It probably is best not to read across. And there's some isomeric issues there, and that's exemplified in the fact that the metabolism of the two is quite different. The 2-Nitro has a lot of triamine formation, which means that the nitro group is reduced; whereas the 4-Nitro it's not. There's very little triamine form. But you've got plenty of data so you don't need to read across. But you really can't use read across here.

DR. ROSS: Oh, I agree. Read across group looked at this briefly, and it was a very quick discussion you've got to split them. You can't read across. So you know, it's maybe you split these out and have individual reports just like every other substituted amine that we've dealt with.

DR. SAM COHEN: As far as the toxicity goes, do you want to cover that?

DR. DAVID COHEN: I think, let's review both of them.

DR. SAM COHEN: Yeah.

DR. DAVID COHEN: If we can't read across do we still need to split the reports?

DR. ROSS: Well, every other one you've dealt with has been a single report for a single compound. I don't really see why this one should be any different.

DR. DAVID COHEN: Yeah, and we've done that with the other hair dyes, too. Remember when we had that amalgamation of about four or six of them. And we did it for two years?

MS. BURNETT: You got the last one on the docket.

DR. DAVID COHEN: Yes, the last one. That's right. So what are our data needs?

DR. BERGFELD: Could I just ask a question? It's Annex II or Annex III? Both are in here.

MS. BURNETT: One's on Annex II. The 2-amino is on Annex II.

DR. ROSS: And 4-amino is on Annex III, Up to 0.5 percent.

DR. DAVID COHEN: 2 is on 2.

DR. TILTON: I mean, that is prohibited, but it says likely because there's no supporting information, it's because there's no use.

DR. ROSS: Well, in the interest of time, you know, I went through this and at least my sense of it was that the 4-Nitro -- sorry about this -- but in individuals that are non-sensitized, I thought 4-Nitro was safe as used in oxidative hair dyes under the current conditions, which was a 0.075 percent max. That's not in the Conclusion. Current conditions is in the Conclusion.

The 2-Nitro I thought was insufficient. I thought it needed a maximum use concentration, sorry. We don't have a margin of exposure calculation on the 2-Nitro, whereas, we do on the 4-Nitro. In fact, we do on most of the hair dyes we've done.

And so, I felt we needed a NOAEL complete with a dose-response relationship. Because I can pull out some dermal tox data. So you've got some dermal tox NOAELs in here but there's no dose response, so that's not helpful.

We need phototoxicity and photosensitization data. We have that on the 4-Nitro. I had a question in there, actually. Does DC -- which stands for David Cohen -- does DC think the 4-Nitro phototox data is appropriate? But anyway, you can comment on that in a minute.

So maximum use concentration, suitable study for a NOAEL, phototox, photosensitization data, and obviously the comment on it being a skin sensitizer, which would be anyway. So that was 2-Nitro.

DR. DAVID COHEN: If we're going to split them, we might as well line up the IDAs now.

DR. ROSS: Yep.

DR. DAVID COHEN: What do you have, Susan?

DR. TILTON: Actually, very similar. So for 4-nitro, concluding safe as used when non-sensitizing. I also listed the limit for under-oxidative formulation due to high dermal absorption.

DR. DAVID COHEN: What was the comment? I mean, can you reiterate that?

DR. TILTON: Safe as used as a hair dye when non-sensitizing under oxidative formulations.

DR. DAVID COHEN: We don't use non-sensitizing for hair dye. That's for plants, right? So it can't be formulated to be non-sensitizing because it's sensitizing, right? So it's safe as used as a hair dye with a patch-test warning, right?

DR. TILTON: Okay. As an oxidative hair dye.

DR. DAVID COHEN: Okay.

DR. TILTON: And for 2-Nitro I noted max concentration of use and phototoxicity. And I would agree on an MOE, having noted that. But that is something that we typically included.

DR. DAVID COHEN: Sam?

DR. SAM COHEN: I agree with that completely. I think the tox data is very clean. Even though there's some genotox on some of these, the carc data is negative. On the 4-Nitro, there's some evidence of liver potential (inaudible) including a thyroid effect, but that's almost for sure CAR (phonetic) activation is not relevant to humans.

The tumorgenicity is essentially negative. And, you know, data needs, as David pointed out, (inaudible).

DR. DAVID COHEN: Do we have the spectral absorption for the two?

DR. ROSS: I don't believe so.

MS. BURNETT: No.

MS. FIUME: Phototoxicity study.

DR. ROSS: You had phototoxicity data in fibroblasts for 4-Nitro?

MS. FIUME: In Table 5, PDF Page 32.

DR. DAVID COHEN: Table 5. Phototox. That's for the 4?

DR. ROSS: For the 4, yeah.

DR. DAVID COHEN: But not for the 2?

MS. BURNETT: Correct.

DR. ROSS: And that was fibroblasts 100 micrograms per milliliter.

DR. DAVID COHEN: So we need it for the 2. I'm just backing up on why we needed it to begin with. Was it just standard practice?

DR. BERGFELD: What, the phototox?

DR. ROSS: Well, we certainly have it for one.

DR. BERGFELD: There has to be some reason here.

DR. DAVID COHEN: Well, that's why I paused when David handed me the baton on that.

DR. ROSS: Well you're going to have absorption with these things, looking at the structure of it.

DR. DAVID COHEN: I'm just doing a Google search. It says it absorbs light particularly in visible near UV regions.

MS. FIUME: So it's PCPC that the data came from, from Europe.

DR. DAVID COHEN: For which? For 2?

MS. BURNETT: For 4.

MS. FIUME: For the 4, yeah.

DR. DAVID COHEN: I'll go back and try to look for something there. I mean, we can ask for that. Yeah. Okay. So maybe we ask for spectral absorption data, and if in the UV range, we ask for phototox. Is that fair?

DR. ROSS: Yeah. It will be in the UV range.

DR. SAM COHEN: Yeah. These almost for sure will be absorbing. So I would just ask for the phototox.

DR. DAVID COHEN: Okay. So, we're going right to phototox. Thank you. Thank you, chemists.

DR. ROSS: Exactly.

DR. DAVID COHEN: Any other comments? Okay. So we're going safe as used for 4, IDA for 2, and we're splitting the reports.

MS. FIUME: So, clarifying. Safe as used in oxidative hair dyes?

DR. DAVID COHEN: Yeah.

MS. FIUME: Okay.

Full Panel – June 10, 2025

DR. DAVID COHEN: Thank you. This is a Draft-Amended Report on the safety of 2-Nitro-p-Phenylenediamine and 4-Nitro-o-Phenylenediamine. First published in 1985, the Panel concluded that the two are skin sensitizers in Guinea pigs. And information in this report and the report on P Phenylenediamine suggests that they have the potential for human sensitization. For those persons not sensitized, the expert Panel concludes that 2- and 4-Nitro compounds are safe as hair dye ingredients at the current concentration of use.

The Panel previously considered a re-review of the report in 2003, and reaffirmed the 1985 conclusion as published in 2006. At the December 2024 meeting, the Panel reopened the safety assessments for these ingredients to reevaluate the data on 2-Nitro-p-Phenylenediamine, especially since it's been categorized as EC Annex II, the published substance prohibited in cosmetic products.

There was no SCCS opinion and this categorization was presumed to be no reported use. While these Nitro-Phenylenediamine ingredients are positional isomers, structural activity relationships between chemicals with different substitution patterns around the aromatic ring are equivocal at best, therefore, read across would be challenging.

Since December 2024, the only new data available has been updated concentration of use survey by the Council. According to the FDA RLD, in 2024, 2-Nitro-p-Phenylenediamine is reported in three hair coloring preparations. No concentration of use was reported in the 2025 Council survey, whereas the Council's 2003 survey reported a max concentration of 1 percent. For 4-Nitro-o-Phenylenediamine, the RLD reported 143 uses. In 2025, the Max concentration decreased to 0.075 percent and hair dyes and colors.

Our group concluded that we cannot read across, the metabolism of the two are different. Our motion was as follows: to split the reports and not split the decisions. For a split report on 4-Nitro, we were safe as used as an oxidative hair dye. For 2-Nitro we would issue an Insufficient Data Announcement requiring maximum concentration of use, phototox and photosensitization, and we don't have a MOE. I think we have a request for a NOAEL and a dose-response relationship. David, did I articulate that? Yeah. Okay, that's our motion.

DR. BELSITO: What do you want the MOE on?

DR. DAVID COHEN: You mean what's the endpoint?

DR. BELSITO: Yeah. What was your concern for the 2-Nitro?

DR. ROSS: Don, when we were considering the 4-Nitro and the 2-Nitro, the 4-Nitro had a MOE in there, the 2-Nitro didn't, so we were just looking at it in comparative terms. And usually when we do these -- in fact, most of the hair dyes we've done have had MOEs associated with them. So those two observations.

DR. BERGFELD: Any comments or a second?

DR. BELSITO: I'm fine with the insufficiency because we don't know the concentration of the 2-Nitro, which is probably the basis for what was done in Europe. That there was no concentration given and no data for it. That's sort of the way they operate. So, if you want to add the margin of exposure, but again I think we need to tell them what endpoint we want them to use.

DR. SNYDER: We were in alignment with that conclusion with one minor detail. We actually wanted to also have skin absorption as in formulation.

DR. BERGFELD: In which one?

DR. SNYDER: For the 2-Nitro. And if absorbed then we would want additional data. That's the only minor change that our team would add to that. Is that correct, Don?

DR. BELSITO: Yes.

DR. DAVID COHEN: That would help with your MOE anyway, right?

DR. SNYDER: Yes.

DR. ROSS: Yeah, the one done for 4-Nitro, Don, was on a 90-day chronic study.

DR. BERGFELD: So are you seconding the motion and just that editorial?

DR. SNYDER: If he makes that minor addition, yes.

DR. DAVID COHEN: Yes, I would amend the motion for 2-Nitro to include dermal absorption.

DR. SNYDER: Okay. Second.

DR. BERGFELD: Good. Any other comment?

DR. BELSITO: But we did have some dermal absorption in humans on PDF Page 20, at the top of the page.

DR. DAVID COHEN: Hold on, Don, I'm just pulling it up.

DR. BELSITO: It's from the old report.

DR. DAVID COHEN: Dermal --

DR. SNYDER: Does that go hand in hand, Don, with that we don't have concentration of use? So if it's higher than previously reported then we would want --

DR. BELSITO: Right. Yeah, I mean, I agree. We also have dermal for 2-Nitro in three monkeys. That's on PDF Page 19. But, yeah, I think we definitely need concentration of use.

DR. DAVID COHEN: Is he arguing against your amendment or not?

DR. SNYDER: So you don't want to include the absorption like we did yesterday in our Panel? We said the three things we wanted were the maximum concentration of use, skin absorption as used in formulation, and the photosensitization, the three needs for 2-Nitro. So you're okay with the absorption data now that you relook at it?

DR. BELSITO: Yeah, I was.

DR. SNYDER: Okay. All right.

DR. DAVID COHEN: We're back to my original.

DR. SNYDER: Back to the original motion. Second.

DR. BERGFELD: Okay. Any other comments about this IDA? Seeing none, I'm going to call the question. It looks like the 4 is safe and the 2 has Insufficient Data Announcement. Is that everyone's perception? We call that to question?

DR. BELSITO: Right. And we're splitting them into two separate reports.

DR. BERGFELD: And we're splitting them into two different reports, correct. All right. All those in favor? Yes, Bart?

DR. HELDRETH: I'm sorry. And I think Dr. Ross was going to clarify what's the endpoint for the MOE.

DR. DAVID COHEN: Oh, the DART -- the MOE? Yeah.

DR. ROSS: Well, first of all, I think we concluded way back we don't need MOEs on everything. So we don't have to do them. But, when you compare the 4-Nitro and the 2-Nitro, side by side, when they're in one report, you know there was a clear absence of the MOE with the 2. That's why we were doing that.

I still think it would be useful. So if we're going to ask for it, you would go with a 90-day chronic study, which was done on the 4-Nitro.

DR. SNYDER: I think we'd be okay with that because the 4-Nitro has low dermal absorption compared to the 2-Nitro. I think it makes sense.

DR. DAVID COHEN: Yeah. I think having the MOE on this group of chemicals has intrinsic value.

DR. ROSS: No argument here.

DR. BELSITO: I may have misunderstood you. So you're going to use the 4-Nitro to calculate the margin exposure for the 2, David?

DR. ROSS: Absolutely not. No. Not at all.

DR. BELSITO: Which one are you using?

DR. ROSS: It was just when they were together in the reports, the lack of an MOE for the 2 was clearly apparent because you had them in the same report.

DR. BELSITO: I see.

DR. ROSS: Now just because we've split the reports, it doesn't necessarily mean that you shouldn't have an MOE for the 2. I think it would really benefit the report and benefit our decision if we had it. So I think it's appropriate to ask for it. If you're going to ask for it, I would use the same data that was used for the 4-Nitro to try and generate that, or trying to come up with that. And if you look back that was a 90-day chronic study that was used for a NOAEL.

DR. BERGFELD: Any further statements or comments before we get the question called? Seeing none, I call the question then. All those in favor of what's been said, please indicate by raising your hands. Is Don raising? Yeah, unanimous. Okay.

SEPTEMBER 2025 PANEL MEETING – DRAFT TENTATIVE AMENDED REPORT

Belsito Team – September 8, 2025

DR. BELSITO: Move on to 2-Nitro-*p*-Phenylenediamine. Okay. So, I'm not going to reiterate all of what went on in '85, but we reopened this in 2024 and decided in June of 2025 to split the 2-Nitro-*p*-Phenylenediamine out of the 4-Nitro-*o*-Phenylenediamine report. And, at that meeting we thought that the data were insufficient to support the safety of the 2-Nitro-*p*-Phenylenediamine.

And the insufficient data were maximum concentration of use in hair dye, 90-day oral repeated dose study with a NOAEL that shows a dose response. And I don't know why we asked for phototoxicity photosensitization. Since it's a hair dye, irritation and sensitization is exempt.

Anyway, we haven't received any new data, so I would go insufficient for maximum use concentration in the 90-day oral with the dose response. I guess we can keep in photo sensitivity, photo irritation, but I really think that it's covered by the sensitization exemption of coal tar hair dyes.

DR. SNYDER: I agree with that and second that motion.

DR. KLAASSEN: Yeah. It's fine.

DR. BELSITO: So, I guess we can discuss tomorrow whether the Cohen team wants to keep photo sensitization, photo irritation in there. Plus, these would be consumed so quickly. I don't think there's going to be anything left to get excited by light. But okay. Any other comments on 2-Nitro-*p*?

MS. BURNETT: So, is the Discussion as written okay? Do you have any other additions you'd like?

DR. BELSITO: I thought it was fine. I didn't have any comments.

MS. BURNETT: Okay. Thank you.

Cohen Team – September 8, 2025

DR. DAVID COHEN: I'm going to go to --

DR. BERGFELD: 2-Nitro.

DR. DAVID COHEN: Yeah. So, this is a Draft Tentative Amended Report on the safety of 2-Nitro-*p*-Phenylenediamine. This was split out as I mentioned in the prior report. The Panel determined that data were insufficient to support the safety of 2-Nitro-*p*-Phenylenediamine as a hair dye ingredient. The IDA had the following data needs: maximum concentration of use in hair dye formulations, a 90-day oral repeated dose study with a NOAEL, phototox and photosensitization data. And since the issuance of the IDA, we've received no additional data. So, this will go out as an Insufficient Data Conclusion.

DR. EISENMANN: I do have a request that the language of the second data need be revised. Reading this report over again, there's just so much animal data already in it, but none of the studies have milligram doses calculated. I think if somebody went carefully through the studies and calculated some milligrams per kilogram, we might not need another 90-day study.

So, to me if you could just -- I don't want anybody to do it because you still don't have the first data need, the concentration of use, but if you could somehow adjust the language to say, I don't know, to calculate, to review the existing animal data to determine if a NOAEL could be determined, and if not, then you need a 90-day study, something like that. I mean, there's an old NCI bioassay, there's tables and tables of the non-carcinogenic endpoints. They just did not summarize it in any text.

There's also some dermal studies. The doses aren't given as milligram per kilogram doses, they're given as, I think, milliliters of the dose material. If you calculate a milligram per kilogram dose, you might be able to get a NOAEL out of them. That would be useful.

DR. ROSS: No, we did look at that. I mean, we did look at that, and I think one of the key issues, Carol, there's no dose response we could see. And so, it wasn't just coming up with a dose, it was looking at a dose response. I forget how the request was worded in that call, but yeah.

And I seem to remember this was just split out from the four. The combination this was in with the four study, and there was an MOE in there and then we just divided them out and wanted an MOE in here, and we couldn't find a NOAEL value that we had confidence in, that had came from a dose response study.

DR. EISENMANN: But none of the values -- I mean, they're like ppm in the diet. I don't know if you actually calculated some doses and I looked at the tables in the NCI study, because -- I think they did two doses rather than three.

DR. ROSS: Yeah, it's hard to get a dose response from that, but they did 1,100 and 2,200 ppm in rats. And I'm seeing some tumor readouts by the way, and 4,400 ppm in mice. So, yeah, you can do that. But, again, it's not a dose response.

DR. EISENMANN: But even if you had a freestanding NOAEL that was much, much higher than the exposure, that might be helpful. I just think there was just so many -- like there's a dermal study where there -- I don't know, three times a week for 90 days or something like that. I just -- I would like to say -- I don't think anybody's going to do it. I think some of the animal data is worth taking a more detailed look, because there's just so much of it in there.

DR. ROSS: Yeah, I mean, I remember going through that and a lot of it was on DART, I seem to recall. Which you can use. Yeah, I mean, sub-chronic, the dermal 0.55 and 1.1 percent.

DR. EISENMANN: Right, none of it says milligram per kilogram per day.

DR. ROSS: Yeah, you can convert it. And they come up with NOAELs, but they only use one dose, so, I mean, that's the problem. I mean from a toxicological standpoint, you can't really --

DR. EISENMANN: But if you converted them all and had a total look at all as a dose level, I think it might be a worthwhile exercise before you did a 90-day study.

DR. ROSS: Well, maybe, but just going through that data, if you've got one or two values, you're not going to get a dose response, and you're not going to get a NOAEL of any confidence. That's (inaudible). I mean, you can ask Jinqiu to go through the data and see if he can see something.

DR. EISENMANN: No, no, I don't think he should do it because you still don't have the concentration of use information. All I'm saying is that you're going directly to asking for this additional 90-day study, and I'm not convinced that you've completely -- I would like to say to take a look at the existing animal data and then consider a 90-day study, based on that information.

DR. ROSS: From the request --

DR. EISENMANN: I don't know. I just think you can just use the animal studies a little bit better than what has been done. But I'm not suggesting you do it at this point because you're still insufficient for concentration of use, and you're not going to get that.

But I just -- in this day and age of nobody wanting to do another animal study, to give somebody a little hope that, yeah, maybe if they a really careful look at some of these studies and the NCI data tables, on the non-cancer endpoints, it might not have to do a 90-day study.

DR. ROSS: I hear what you're saying and maybe we can rephrase that, but I don't think you're going to get what you want from the existing data, because we did look at that. With respect to how you rephrase that, you could just say -- instead of a lack, you could say provision of the suitable NOAEL from a 90-day study with a dose response, and that could come from -- you know, it doesn't have to come from a new study.

DR. EISENMANN: Okay. That's fine.

DR. DAVID COHEN: That would be a new IDA, because isn't this -- this is a Draft Tentative Report.

DR. EISENMANN: Yeah. No, it doesn't -- I don't think it needs to be -- to me, it's just wordsmithing the second one to somehow acknowledge some of the animal data that's already there, even if it's using it to help design a new study. But there's just so much there that --

DR. ROSS: You may be able to get NOAELs, but you're not going to get a dose response from the data that's in there.

DR. EISENMANN: I understand that. But if there's a large gap already between the existing NOAELs and the exposure, then you might be okay. You're not going to get exposure because we don't have a maximum concentration of use, which is why I don't want you to actually do the exercise because there's no use spending the time.

DR. DAVID COHEN: So, (audio skip) concentration of use, the IDA won't be satisfied, you know, I mean this only in the nicest way, why are we having this conversation if we know the IDA is not going to be met?

DR. EISENMANN: Just because of the animal issue. Because there's so many animal studies already in there and here you're asking for another animal study. I think it just might be helpful to, you know -- I don't know. It just struck me as I read the report again, how much animal studies were in that report, and then here's another animal study.

DR. ROSS: No, I take your point. And I think it just came from a direct comparison with the 4-Nitro that was in the previous report together. And I think, David, you could perhaps change the wording, in that it would be less restrictive in a sense, because your wording might go to provision of a NOAEL with a dose response to enable an MOE calculation.

DR. DAVID COHEN: Do you want me to add the word after dose response relationship to, "in order to calculate an MOE?"

DR. ROSS: How did you raise the insufficiency?

DR. DAVID COHEN: IDA (audio skip) a 90-day oral repeated dose study with a no observable adverse effect level that shows the dose response relationship.

DR. ROSS: Well, I think that's the same thing we were saying, Carol. I think it doesn't have to be a new one, it could be provided from existing.

DR. EISENMANN: Right.

DR. ROSS: I think that's fine.

DR. SAM COHEN: Why don't we just ask for data that could provide a NOAEL and dose response study for calculating an MOE, without asking whether it's a 28-day study or whatever study.

DR. ROSS: Or it's a DART study or whatever, yeah.

DR. SAM COHEN: Yeah. David, let me ask you, why would you need a dose response study to do an MOE? Couldn't you just rely on the NOAEL?

DR. ROSS: Yeah, but to get the NOAEL you need a dose response.

DR. SAM COHEN: No. If you have a study that has a no effect level, that's the no effect level, regardless of what doses is above that, caused an effect.

DR. ROSS: Yeah, but to get one that's going to give you some confidence in the actual MOE, you need a dose response.

DR. SAM COHEN: Now you're getting picky.

DR. ROSS: No, you do. I mean, it's just the way it is. But I have a question for you, Sam, and this gets at the one we've also just done, the 4-Chloro-Aminophenol.

There's 2-Nitro -- I mean, we've got genotox with this one. It's AMES positive, right. And we've got carcinogenicity data with the 2-Nitro-Phenylelenediamine. Now that was disputed by two independent pathologists, but I'm just trying to understand how would this one -- and also this one we've got extensive absorption. So how does it differ from the 4-Chloro-2-Aminophenol we just did and declared as unsafe?

DR. SAM COHEN: I think the main thing here is that you don't really have an animal-positive tumor data. The mouse study is clearly negative, and the rat study basically is no tumor increased incidences of above background, especially if you take into account historical controls. There was nothing in the dog study that suggested and proliferative.

And the genotox, there was a kind of a mix. But you're right, I think overall the genotox would favor that it is positive and one would have concerns about this, but I don't think you had the tumor data like you do with Chloro-Phenol one.

DR. ROSS: Well, that was bladder tumors at high dose, right? This one was adenomas and carcinomas at 4,400 parts per million, which is 0.44 percent.

DR. SAM COHEN: But that wasn't in the bladder.

DR. ROSS: It wasn't in the bladder, but conclusion was that it was carcinogenic to female mice. That was the conclusion. And then I wanted your take on this, two independent pathologists reviewed the data and disputed that.

DR. SAM COHEN: Yeah, I think that in those old studies, in mice with the classification of tumors, there was a lot of controversy as to what was the true adenoma and what was the carcinoma, and many of them actually are just hepatic foci that are basically hyperplasia. And then on top of it, there's not a lot of confidence in mouse liver as an actual marker for predicting human carcinogenicity. In fact, until the ICH came into existence, Europe didn't require a mouse study at all.

DR. ROSS: And then in rats we put 1,100 and 2,200 parts per million giving some evidence of cancer, but I didn't quite understand this.

DR. SAM COHEN: I didn't think it was significant.

DR. ROSS: The conclusion was there was no positive -- significant associations between 2-Nitro-Phenylenediamine and some types of cancer, but overall there was no convincing evidence.

DR. SAM COHEN: Yeah. And that's because I think none of them were statistically significant. And if you go back to the mouse data with the liver tumors, you had one with the classification that makes the whole thing uninterpretable. But even so they didn't take into account historical controls. This was an older study that didn't consider historical controls as much as we do today.

Plus, the level of significance for common tumors -- and common tumors is defined as those with a background incidence of greater than one percent, which certainly liver tumors in the mice is well above that. That the level of significance has to be at P less than 0.01, not 0.05, and that would not even come close.

DR. ROSS: So, you're confident drawing a distinction between the 4-Chloro-2-Aminophenol that we've just done and found unsafe, versus this one?

DR. SAM COHEN: Yes.

DR. ROSS: Okay, that's all I need to know.

DR. BERGFELD: David?

DR. DAVID COHEN: Okay. Let's move on to Butoxyethanol. This is a draft amended --

DR. SAM COHEN: Just let me finish up one more comment on the last one, is that it's been basically -- it's going to be ended for non-use and lack of providing us data. So, I don't think we can come out as definitively saying that there's a cancer risk or genotox risk, like we could with the other one where it was pretty clear cut.

DR. ROSS: I think the genotox looks clear to me, but I think the cancer risk, I take your points. Yeah.

DR. SAM COHEN: Yeah.

Full Panel – September 9, 2025

DR. BELSITO: Yes, so 2-Nitro-*p*-Phenylenediamine. As I discussed with 4-Nitro-*o*-Phenylenediamine was part of a combined report with the ortho in '85. The conclusion was reaffirmed in 2006. But in June of 2025, we decided that we couldn't really read across so we would split them into two.

Subsequently, at that meeting we determined that the data were insufficient to support safety of 2-Nitro-*p*-Phenylenediamine as a hair dye ingredient. And an IDA was issued for maximum concentration of use in hair dye formulations, a 90-day oral repeated dose with an NOAEL that shows dose response. And I don't know why we added phototox/photosensitization, but we did, because sensitization/irritation is not an issue with hair dyes.

We got no new data, so we would agree insufficient. And that's my motion, although I would drop the phototox/photosensitization from our needs.

DR. BERGFELD: And that's a motion. And that's seconded?

DR. DAVID COHEN: Yup.

DR. BERGFELD: Any further discussion or edits of this particular motion or document? Seeing none, all those disapproved? Abstained? This is approved and moving forward as insufficient.

DECEMBER 1983 PANEL MEETING – ORIGINAL REVIEW

Closed Session of the Expert Panel Meeting and Chairman's Summary

Dr. Beyer called a closed session of the Expert Panel meeting for the purpose of discussing whether the three reports on hair dye ingredients (*p*-Phenylenediamine, 2-Nitro-*p*-Phenylenediamine/4-Nitro-*o*-Phenylenediamine, and Disperse Black 9) should be combined into a single document with a common conclusion. It was generally agreed that each ingredient is chemically distinct and should be handled separately. Dr. Boutwell suggested that the three reports be published together. Dr. Bergfeld suggested that an Introduction be written to express concerns common to all the ingredients and explain why they are handled as separate documents.

Dr. Beyer opened discussion as to whether to incorporate the cautionary statement required on the label into the Conclusion of the reports. The general consensus was that reference should be made to it in the texts and/or Discussion Sections, but not in the Conclusions. However, no final decision was reached.

Full Panel

The Panel discussed the carcinogenicity data on these two compounds, and found them to be equivocal. Dr. Bergfeld stated that reference would have to be made in the text to the *p*-Phenylenediamine document with regard to carcinogenesis, mutagenesis, and teratogenesis. She also stated that the clinical data in this report are weak, except for the RIPT studies done on 206 individuals on both these and the p-Phenylenediamine compounds.

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

> "2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine are skin sensitizers for guinea pigs. Information in this report and in the report on p-Phenylenediamine suggests that 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine have potential for human sensitization. For those persons not sensitized, the Expert Panel concludes that 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine are safe as hair dye ingredients at the current concentrations of use."

Subject to minor revisions, and pending receipt of Dr. Hoffmann's edited version of the Carcinogenesis Section, the document will be announced as a Tentative Report.

NOVEMBER 2003 MEETING – FIRST RE-REVIEW

Full Panel – November 14, 2003

Dr. Belsito noted that a CIR Final Report with the following conclusion on these ingredients was published in 1985: 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine are skin sensitizers for guinea pigs. Information in this report and in the report on *p*-Phenylenediamine suggests that 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine have potential for human sensitization. For those persons not sensitized, the Expert Panel concludes that 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine are safe as hair dye ingredients at the current concentration of use.

Dr. Belsito also noted that, in 1993, the International Agency for Research on Cancer (IARC) published a report on 2-Nitro-*p*-Phenylenediamine (a.k.a. 1,4–Diamino-2-Nitrobenzene) with the following overall conclusion: 1,4-Diamino-2-Nitrobenzene is not classifiable as to its carcinogenicity to humans. He added that, in its prior review of 2-Nitro-*p*-Phenylenediamine, the Expert Panel discussed the data included in the IARC report and addressed the points that were made by IARC in this report. After reviewing the IARC report plus the other data on 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine that were presented in the re-review document, Dr. Belsito said that his Team concluded that the Panel's published safety assessment should not be reopened.

Dr. Marks recommended that the Panel's new hair dye epidemiology statement be included in the entry for 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine in the Annual Review.

The Expert Panel unanimously concluded that the Panel's Final Safety Assessment on 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine should not be reopened.

Amended Safety Assessment of 2-Nitro-*p*-Phenylenediamine as Used in Cosmetics

Status: Draft Final Amended Report for Panel Review
Release Date: May 22, 2026
Panel Meeting: June 15-16, 2026

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

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ABBREVIATIONS

ADME	absorption, distribution, metabolism, and excretion
BHA	butylated hydroxyanisole
CHO	Chinese hamster ovary
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
Dictionary	*International Cosmetic Ingredient Dictionary*
DMSO	dimethyl sulfoxide
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FD&C Act	Food, Drug, and Cosmetic Act
HPLC	high-performance liquid chromatography
IARC	International Agency for Research on Cancer
MoCRA	Modernization of Cosmetics Regulation Act
MOE	margin of exposure
MOS	margin of safety
NOAEL	no-observed-adverse-effect level
NR	not reported
Panel	Expert Panel for Cosmetic Ingredient Safety
PII	primary irritation index
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RLD	Registration and Listing Data
SCCS	Scientific Committee on Consumer Safety
SCCNFP	Scientific Committee on Cosmetic Products and Non-Food Products
SED	systemic exposure dose
$T_{1/2}$	half time
US	United States
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) reassessed the safety of 2-Nitro-*p*-Phenylenediamine, which is reported to function as a hair dye in cosmetic products. The Panel reviewed all relevant data related to this ingredient. The Panel issued an amended report with a revised conclusion stating that the available data are insufficient to make a determination of safety for 2-Nitro-*p*-Phenylenediamine under the intended conditions of use as a hair dye ingredient.

INTRODUCTION

2-Nitro-p-Phenylenediamine, which according to the *International Cosmetic Ingredient Dictionary* (*Dictionary*) is reported to function as a hair colorant,[1] was previously reviewed by the Panel as part of a safety assessment with 4-Nitro-*o*-Phenylenediamine that was first published in 1985.[2] At the time, the Panel concluded:

> *[2-Nitro-p-Phenylenediamine] and [4-Nitro-o-Phenylenediamine] are skin sensitizers for guinea pigs. Information in this report and in the report on [p-Phenylenediamine] suggests that [2-Nitro-p-Phenylenediamine] and [4-Nitro-o-Phenylenediamine] have potential for human sensitization. For those persons not sensitized, the Expert Panel concludes that [2-Nitro-p-Phenylenediamine] and [4-Nitro-o-Phenylenediamine] are safe as hair dye ingredients at the current concentration of use.*[2]

The Panel previously considered a re-review of this report in 2003[3] and reaffirmed the 1985 conclusion, as published in 2006.[4]

In accordance with its Procedures, the Panel re-evaluates the conclusions of previously issued reports every 15 years, and as it had been at least 15 years since the previous re-review was issued, the Panel again considered a re-review of these ingredients at the December 2024 meeting. At that meeting, the Panel determined that this safety assessment should be re-opened to re-evaluate the data on 2 Nitro-*p*-Phenylenediamine, especially since it has been categorized by the European Commission on Annex II, the list of substances prohibited in cosmetic products.[5] Furthermore, the Panel determined that data for the two nitro phenylenediamine hair dye ingredients included in the original report could not be read across; accordingly, rather than presenting 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine together in one amended report, re-reviews of each hair dye have been developed as individual stand-alone reports.

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; a search was last conducted April 2026. 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.

Excerpts from the summaries of the 1985 report and the 2003 re-review document are disseminated throughout the text of this document, as appropriate, and are identified by *italicized text*. (This information is not included in the tables or the Summary section).

Some of the chemical and toxicological data on 2-Nitro-*p*-Phenylenediamine 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, Authorisation and Restriction of Chemicals (REACH) chemical registration process.[6] These data summaries are available on the database for ECHA, and when deemed appropriate, information from the summaries has been included in this report.

CHEMISTRY

Definition and Structure

2-Nitro-*p*-Phenylenediamine (CAS No. 5307-14-2) is the substituted aromatic amine that conforms to the structure in Figure 1:[CIR Staff,1]

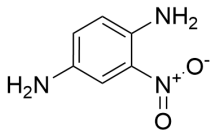

Figure 1. 2-Nitro-*p*-Phenylenediamine

This ingredient is reported to function as a semi-permanent and permanent hair colorant in cosmetic formulations.[1,2]

Chemical Properties

Chemical properties are summarized in Table 1. 2-Nitro-*p*-Phenylenediamine is reported to be a greenish to brownish crystalline powder with a molecular weight of 153.14 g/mol and a density of 0.769 g/ml at 20 ºC.[6] The estimated log K_{ow} is 0.53.

Method of Manufacture

2-Nitro-p-Phenylenediamine may be prepared by the hydrolysis of 1,4-diamino-4-acetyl-2-nitrobenzene.[2] *It may also be prepared by acetylating p-phenylenediamine with acetic anhydride, followed by nitration and hydrolysis*

Impurities

2-Nitro-p-Phenylenediamine has a minimum purity of 97 - 99%.[2] *It may contain a maximum of 100 ppm iron and nitroaminoacetanilide isomers as impurities.*

USE

Cosmetic

The safety of the cosmetic ingredient 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 2-Nitro-*p*-Phenylenediamine in cosmetics. Registration and Listing Data (RLD) obtained from the FDA report frequency of use, and responses to a survey conducted by the Personal Care Products Council (Council) indicate maximum reported concentrations of use; it is these values that define the present practices of use and concentration that are assessed by the Panel. Since 2024, as a result of the Modernization of Cosmetics Regulation Act of 2022 (MoCRA), manufacturers and processors are required to register facilities and list their products (and ingredients therein) with the FDA (i.e., RLD). An exception is made for small businesses (average gross annual sales in the US of cosmetic products for the previous 3-yr period is less than $1,000,000, adjusted for inflation), which are exempt from MoCRA reporting for most cosmetic product categories. Eye area products, injected products, internal use products, or products that alter appearance for more than 24 h, and the facilities that manufacture these products, are not included in this exemption.[7] Another change resulting from MoCRA is the addition of tattoo preparations (permanent tattoo inks, temporary tattoo inks, and other tattoo products) to the product categories for which companies need to list their products with FDA. However, evaluating the safety of ingredients as used in tattoo preparations is not within the purview of the Panel; accordingly, such use is not included as part of the present practices of use that are assessed by the Panel.

According to RLD obtained from the FDA in 2025, 2-Nitro-*p*-Phenylenediamine is reported to be used in 4 hair dyes and colors.[8,9] No concentrations of use were reported in the Council's 2025 survey.[10]

This ingredient is considered a coal tar hair dye for which regulations require caution statements and instructions regarding patch tests in order to be exempt from certain adulteration and color additive provisions of the US Federal Food, Drug, and Cosmetic Act (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.

It is possible that some products containing 2-Nitro-*p*-Phenylenediamine may be marketed for use with airbrush delivery systems. With the advent of MoCRA and the current product categories outlined therein, it is now mandatory that cosmetic products used in airbrush delivery systems be reported as such for some, but not all, product categories in the RLD. In other words, a reliable source of frequency of use data regarding the use of cosmetic ingredients in conjunction with airbrush delivery systems is now available, in some instances. The reported product category for this ingredient as listed in the RLD does not include a designation indicating airbrush application, so it is possible that this ingredient is used with airbrush delivery systems, but not reported as such. Additionally, concentration of use surveys are conducted based on product categories as stated in the RLD, but airbrush use was not reported in response to the survey. No consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with airbrush technology, thereby preempting the ability to evaluate risk or safety. Without information regarding the consumer habits and practices data or product particle size data (or other relevant particle data, e.g., diameter) related to this use technology, the data profile is incomplete, and the Panel is not able to determine safety for use in airbrush formulations. If this ingredient was to be used in airbrush formulations, the data are insufficient to evaluate the exposure resulting from cosmetics applied in such a manner.

European regulations regarding cosmetic ingredients categorize 2-Nitro-*p*-Phenylenediamine in Annex II, the list of substances prohibited in cosmetic products.[5] An opinion on 2-Nitro-*p*-Phenylenediamine by the Scientific Committee on Consumer Safety (SCCS) has not been published. However, in 2003, the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFP) issued an opinion titled "Request for a Re-evaluation of the Hair Dyes Listed in Annex III to Directive 76/768/EEC on Cosmetic Products," which included 2-Nitro-*p*-Phenylenediamine, as well as other hair dye ingredients.[11] This opinion requested a re-evaluation of genotoxicity and carcinogenicity data with regard to the committee's opinion on hair dyes and bladder cancers.

Non-Cosmetic

2-Nitro-p-Phenylenediamine is used for dyeing furs.[2]

2-Nitro-*p*-Phenylenediamine is reported to be used as an agrochemical and pharmaceutical intermediate.[6] Additionally, it is used in chemical synthesis, laboratory chemicals, and dyestuff application.

TOXICOKINETIC STUDIES

Dermal Penetration

In an experiment with a hair colorant base containing ^{14}C-2-Nitro-p-Phenylenediamine (0.5%), groups of female rats were treated with 100 µl of 50% aqueous hair colorant base on 10 cm^2 of skin for 5 min.[2] One group was killed immediately after rinsing with distilled water, one group each was rinsed or not rinsed and a non-occlusive 48-h patch was applied, and one group each was rinsed or not rinsed and an occlusive 48-h patch was applied. Skin penetration was 1.7 µg./cm^2 in the rinsed, nonocclusive patch group; 5.0 µg/cm^2 in the not rinsed, nonocclusive patch group; 4.2 µg/cm^2 in the rinsed, occlusive patch group; and 33.9 µg/cm^2 in the not rinsed, occlusive patch group. This experiment also studied groups of female rats that were treated with 200 µl of the 50% aqueous hair colorant base containing 0.5% radiolabeled 2-Nitro-p-Phenylenediamine for 5 to 30 min before rinsing. After rinsing, the skin was covered with a non-occlusive patch. At 48 h, skin penetration had increased from 3.2 µg/cm^2 for a 5-min contact to 6.1 µg/cm^2 for a 30-min contact. In additional study groups, the same test material was applied to the skin for 10 min, rinsed and then further application was made to one group 1 h later, and two further applications were made to another group at hourly intervals. Skin penetration was 4.8 µg/cm^2 in the single application group, 13.2 µg/cm^2 in the double application group, and 13.7 µg/cm^2 in the triple application group. In a related experiment with the hair colorant base, rats received 200 µl of 50% base containing 0.025 - 0.48% of radiolabeled 2-Nitro-p-Phenylenediamine for 5 min. The base was then rinsed off, and a 48-h non-occlusive patch was applied. Skin penetration increased with increasing 2-Nitro-p-Phenylenediamine concentration and was proportional to the concentration; e.g., 0.1 µg/cm^2 with 0.025% and 3.2 µg/cm^2 with 0.48% 2-Nitro-p-Phenylenediamine. Further testing with clipped and non-clipped skin found the skin penetration after 5 min to be 3.2 µg/cm^2 and 1.6 µg/cm^2, respectively.

The percutaneous absorption of ^{14}C-2-Nitro-p-Phenylenediamine (4 µg/cm^2) in acetone was measured using human and monkey skin.[3,12] Rapid penetration of the test material was observed, with maximum absorption occurring during the first few hours. A comparison of the human and monkey data showed a trend toward increased absorption through monkey skin.

In a study evaluating 2 different in vitro modeling setups (PermeGear cells vs. samples in insert) using EpiSkin™ reconstructed skin, 6 different chemicals were analyzed, including 1 mM 2-Nitro-*p*-Phenylenediamine (> 97% pure; vehicle not reported), which allowed interface diffusion to be checked visually.[13] 2-Nitro-*p*-Phenylenediamine was reported to have one of the highest penetration rates of the 6 chemicals tested. No further details about the rates were provided.

Absorption, Distribution, Metabolism, and Excretion

In Vitro

Under aerobic conditions, 2-Nitro-p-Phenylenediamine (concentration not reported) and its acetylated metabolite, 2-nitro-p-phenylenediamine-N4-acetate, were reduced to their corresponding amines in rat liver subcellular fractions, microsomes, and cytosol.[3] In an absorption and metabolism study, 2-Nitro-p-Phenylenediamine (concentration not reported) was evaluated using fuzzy rat and human skin and rat jejunal tissue. Absorption was measured over 24 h in flow-through diffusion cells. Dosing vehicles were applied to the skin and intestine in the diffusion cells for 30 min. Metabolites were determined using high-performance liquid chromatography. The results suggested that 2-Nitro-p-Phenylenediamine is rapidly absorbed and extensively metabolized in both skin and intestinal tissue. In human and rat skin, metabolites were triaminobenzene and N4-acetyl-2-nitro-p-phenylenediamine. A sulfated metabolite was also observed in rat skin, but not in human skin.

Animal

Dermal

In an absorption study with rats, 100 or 200 µl of ^{14}C-2-Nitro-p-Phenylenediamine in ethanol was applied to a 10 cm^2 area of clipped skin and covered with a protective patch.[2] The female rats absorbed 29% of the applied radioactivity; at 48 h, 40% of that absorbed was in the urine, 53% was in the feces, and 7% was in the carcasses. Male rats absorbed 14% of the applied radioactivity; at 48 h, 34% of that absorbed was in the urine, 61% was in the feces, and 5% was in the carcasses.

Radiolabeled 2-Nitro-p-Phenylenediamine (4 µg/cm^2) was applied topically (3 - 15 cm^2) to rhesus monkeys and white swine for 24 h.[2] The radiolabel was excreted in the urine. The peak rate of urinary excretion of radioactivity for monkeys was 4 - 8%/h; for pigs, the rate was 8 - 12%/h. It was estimated that 29.9% of the applied 2-Nitro-p-Phenylenediamine penetrated the skin of monkeys and 17.7% of the applied material penetrated the skin of pigs during the 24-h exposure period.

In an absorption study using 3 monkeys, ^{14}C-labeled 2-Nitro-p-Phenylenediamine (1.36%) was evaluated under direct conditions.[3,14] Dermal absorption from application to the scalp was quantified via urine assays. The assay results refer mostly to data obtained during the 144-h period following application of the dye. Total dose excretion was 0.551 $\pm$ 0.10%. The half time ($T_{1/2}$) of urinary excretion of 2-Nitro-p-Phenylenediamine was 24 h.

Oral

A semi-permanent hair dye and base composite containing 2-Nitro-p-Phenylenediamine was administered orally to rabbits (19.5 and 97.5 mg/kg/d) and given in feed to rats (1950 and 7800 ppm) and dogs (19.5 and 97.5 mg/kg/d); the animals excreted blue-brown urine.[2] No further details were available. Radioactivity was absorbed and was excreted in the urine and feces of rats that received ^{14}C-2-Nitro-p-Phenylenediamine orally.

Parenteral

^{14}C- 2-Nitro-p-Phenylenediamine (2.6 mg/kg) was administered intraperitoneally or intravenously to rats.[2] Radioactivity was distributed throughout the body, and the greatest percentages were found in the muscle, large and small intestines, and liver. Radioactivity was excreted in the bile, feces, and urine. Nonmetabolized 2-Nitro-p-Phenylenediamine, N^4-acetyl-2-nitro-p-phenylenediamine, and N^1,N^4-diacetyl-2-amino-p-phenylenediamine, and two unidentified metabolites were detected in the urine. In rats that were treated orally or injected intraperitoneally with ^{14}C-2-Nitro-p-Phenylenediamine (concentration not reported) in 5% Tween 80, radioactivity was recovered from the urine and feces but not from expired air. Unchanged 2-Nitro-p-Phenylenediamine, acetylated 2-Nitro-p-Phenylenediamine, sulfate and/or glucuronide conjugates of 2-Nitro-p-Phenylenediamine and of acetylated 2-Nitro-p-Phenylenediamine, and two conjugates with sulfur-containing amino acids were detected in the feces.

The metabolism of 100 mg/5 ml/kg 2-Nitro-p-Phenylenediamine in 2% carboxymethyl cellulose was studied in male rats that were dosed intraperitoneally.[3,15] Urine was collected for 24 h. The major metabolites that were isolated and identified were N^4-acetyl-2-nitro-1,4-diaminobenzene and N^1,N^4-diacetyl-1,2,4-triaminobenzene.

Human

Dermal

In an absorption study using 3 subjects, ^{14}C-labeled 2-Nitro-p-Phenylenediamine (1.36%) was evaluated under direct conditions.[3,14] Dermal absorption from application to the scalp was quantified via urine assays. The assay results refer mostly to data obtained during the 144-h period following application of the dye. Total dose excretion was 0.143 $\pm$ 0.04%. $T_{1/2}$ of urinary excretion of 2-Nitro-p-Phenylenediamine was 24 h.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

Groups of 4 to 8 rabbits received 2-Nitro-p-Phenylenediamine in 10 ml of a permanent hair dye base topically on shaved skin (area not reported) for 24 h.[2] No deaths were observed at doses of 5 g/kg 2-Nitro-p-Phenylenediamine. No further details were available.

Oral

In rats, the acute oral LD_{50} for 2-Nitro-p-Phenylenediamine ranged from 1800 to 3080 mg/kg.[2]

Parenteral

In rats, the acute intraperitoneal LD_{50} for 2-Nitro-p-Phenylenediamine was 348 mg/kg.[2]

Short-Term Toxicity Studies

Dermal

Hair dye composites containing 0.55% 2-Nitro-p-Phenylenediamine (diluted 1:1 with hydrogen peroxide activator) were applied topically at up to 4 g/kg/d to rabbits daily for 20 d.[2] There were no treatment-related signs of toxicity and no significant gross abnormalities at necropsy 14 d after the test period. There were local skin reactions during the test period, but the skin appeared normal by the end of the 14-d observation period following the test period.

Oral

In 4-wk studies, no adverse effects were observed in mice that were fed up to 11,830 ppm 2-Nitro-p-Phenylenediamine in their diet or in rats that were fed up to 6800 ppm.[2] There were no adverse effects when a semi-permanent hair-coloring composite containing 0.24% 2-Nitro-p-Phenylenediamine was fed at a concentration of 7800 ppm for more than 8 wk to rats. (No further details were available.)

Parenteral

In intraperitoneal studies, rats that received 40 mg/kg/d 2-Nitro-p-Phenylenediamine for 5 d or 20 mg/kg/d 2-Nitro-p-Phenylenediamine 3 times/wk for 8 wk had suppressed body weight gains.[2]

Subchronic Toxicity Studies

Dermal

In a study of a hair dye formulation containing 1.1% 2-Nitro-p-Phenylenediamine (mixed 1:1 with 6% hydrogen peroxide), 2 ml/kg was applied topically every third day of gestation for 19 d to pregnant rats.[2] No signs of toxicity were observed to the dams and no irritation was observed to the site of application. The same hair dye formulation was used in a rabbit study where the test material was applied topically 2 times/wk for 13 wk. The material was washed off an hour after each application. No adverse effects were reported. In another rabbit study with the hair dye composite containing 0.55% 2-Nitro-p-Phenylenediamine (diluted 1:1 with a hydrogen peroxide activator), 2 oz of the test mixture was applied to the hair for 30 min and then rinsed with tap water in a procedure that was repeated once every 2 wk for 13 wk. No adverse effects were observed.

Chronic Toxicity Studies

Dermal

A semi-permanent colorant shampoo containing 2-Nitro-p-Phenylenediamine (unspecified concentration, diluted 1:4:5 with water and acetone, 0.4 ml) was applied to the skin of strain A and strain DBAf mice 2 times/wk for 80 wk.[2] *Toxic effects were seen in the DBAf strain: obstructing crystals were seen in the urinary bladder and on the penile skin. The urinary bladder, seminal vesicles, and stomachs were distended, the renal tubules were dilated, and a few mice had chronic gastritis. In another study with mice, a hair dye composite containing 1.1% 2-Nitro-p-Phenylenediamine (diluted 1:1 with 6% hydrogen peroxide) was applied topically for 21 to 23 mo; there were no adverse findings.*

In a 20-mo dermal study, groups of 60 male and 60 female Swiss Webster mice received topical applications of 0.05 ml/kg of a semi-permanent hair dye formulation containing 0.85% 2-Nitro-*p*-Phenylenediamine on the back (clipped skin; 1 cm^2) 3 times/wk.[6] All mice survived until study end. The body weight of the treated animals was reduced by no more than 10% of that of the controls. Incidences of liver hemangiomas, lung adenomas, and malignant lymphomas were no greater than in the controls. No skin tumors were observed at the site of application. The no-observed-adverse-effect level (NOAEL) was determined to be 0.05 ml/kg. No further details were available.

A similar study was performed in groups of 60 male and 60 female Sprague-Dawley rats.[6] The rats received topical applications (0.5 ml//kg) of an oxidative hair dye formulation containing 1.1% 2-Nitro-*p*-Phenylenediamine on the back (clipped skin; 2.5 cm diameter) 2 times/wk for 117 wk. The formulation was diluted in an equal volume of 6% hydrogen peroxide. All rats survived until study end. Mean body weights in the treatment groups were comparable to the controls. No significant increase in the incidence of tumors was observed, and no skin tumors were observed. The NOAEL was determined to be 0.5 ml/kg. No further details were available.

Oral

In a 78-wk study, no adverse effects were observed in mice that were fed up to 4400 ppm 2-Nitro-p-Phenylenediamine in their diet or in rats that were fed up to 1100 ppm.[2] *In a study of a semipermanent hair-coloring composite containing 0.24% 2-Nitro-p-Phenylenediamine administered to dogs for 2 yr, no signs of toxicity were observed with oral doses up to 97.5 mg/kg/d.*

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Dermal

A hair dye formulation containing 1.1% 2-Nitro-p-Phenylenediamine (dilution not described) was applied topically 2 times/wk (0.5 ml) to female mice prior to mating and throughout mating and gestation.[2] *There was no evidence of a teratogenic effect, but there may have been a delaying effect on the ossification process, particularly of the bones of the feet and of the cervical and caudal vertebral centra. Local skin reactions, but no other adverse effects, were observed in a 3-generation study of a hair dye formulation containing 1.1% 2-Nitro-p-Phenylenediamine (mixed with 6% hydrogen peroxide); the dye was applied topically in doses up to 0.5 ml 2 times/wk to rats. Topical application of hair dyes containing 1.1% 2-Nitro-p-Phenylenediamine (1:1 dilution with 6% hydrogen peroxide, 2 ml/kg) every third day during the gestation period of rats did not result in any signs of toxicity to the maternal or fetal animals. A hair dye formulation containing 1.1% 2-Nitro-p-Phenylenediamine (dilution not described) applied topically (2 ml/kg) 2 times/wk to female rabbits prior to mating and throughout mating and gestation caused focal alopecia at slightly higher incidences in maternal animals that were treated during the first 2/3 of gestation; no other adverse effects were observed in the maternal animals. No teratogenicity was observed in the fetal animals; evidence of embryotoxicity was observed.*

Oral

No embryotoxicity or teratogenic effects were observed when a semi-permanent hair-coloring composite containing 0.24% 2-Nitro-p-Phenylenediamine was fed at a concentration of 7800 ppm to male or female rats prior to or during mating, or to pregnant rats on days 6 - 15 of gestation.[2] *Up to 97.5 mg/kg/d of this composite was administered orally to pregnant rabbits on days 6 - 18 of gestation; no signs of toxicity were observed to the maternal or fetal rabbits.*

Parenteral

A significant decrease in maternal weight gain was observed in mice that received 2-Nitro-p-Phenylenediamine (32 to >160 mg/kg/d) subcutaneously on days 6 - 15 of gestation; significant maternal toxicity was observed at doses of 160 mg/kg/d or more.[2] *The number of fetuses with cleft palates and blood vessel malformations was significantly increased at doses ≥ 160 mg/kg/d. Average fetal weight was decreased with ≥ 128 mg/kg/d. Dose-related increases in resorption incidence and number of stunted fetuses were observed with 2-Nitro-p-Phenylenediamine. The highest "no effect" dose of 2-Nitro-p-Phenylenediamine was 64 mg/kg/d.*

GENOTOXICITY STUDIES

In several Ames tests, 2-Nitro-p-Phenylenediamine (up to 6666 µg/plate) was determined to be mutagenic, with and without metabolic activation.[2,3] *2-Nitro-p-Phenylenediamine was not mutagenic in an assay using two strains of Escherichia coli (concentrations tested not reported).*[2] *Hair dyes containing 2-Nitro-p-Phenylenediamine (concentrations not reported) were negative in mutagenesis assays with several strains of E. coli. 2-Nitro-p-Phenylenediamine (up to 500 µg/ml) was*

mutagenic in L5178Y mouse lymphoma assays.[3] *An increase in chromosomal aberrations was observed in a study using CHO cells exposed to up to 738 µg/ml without metabolic activation.*[3] *2-Nitro-p-Phenylenediamine caused chromosome damage in human peripheral blood lymphocytes when tested at up to 100 µg/ml.*[2]

In in vivo studies, 2-Nitro-p-Phenylenediamine was not genotoxic in a chromosomal aberration study in mice (at "0.2 LD_{50} or 0.5 $LD_{50,}$" intraperitoneally), or in micronucleus tests in mice (up to 1000 mg/kg, intraperitoneally) or rats (up to 2000 mg/kg, intraperitoneally).[2,3] *The induction of mutations in the lacl gene was studied in transgenic Big Blue C57BL/6 mice that received 150 mg/kg/d 2-Nitro-p-Phenylenediamine for 10 d over a 2-wk period.*[3] *Compared to vehicle controls, an increased mutant frequency was observed in males by a factor of 2; however, no increases were observed in females. 2-Nitro-p-Phenylenediamine (20 mg/kg/d, 3 times/wk for 8 wk) was negative in a dominant lethal assay in rats.*[2]

In an alkaline comet assay, human lymphocytes were incubated with 50 - 500 µM 2-Nitro-*p*-Phenylenediamine in dimethyl sulfoxide (DMSO) for 2 h.[16] Positive and negative controls were hydrogen peroxide and DMSO, respectively. Genotoxicity as single strand DNA breaks was observed with 2-Nitro-*p*-Phenylenediamine in a dose-dependent manner ($p < 0.05$).

Mutagenicity Enhancement/Inhibition

In the presence of metabolic activation, the mutagenicity of 2-Nitro-p-Phenylenediamine (0.46 µM) was significantly inhibited by butylated hydroxyanisole (BHA) in a study performed in S. typhimurium strain TA98.[3] *Antimutagenic effects on 2-Nitro-p-Phenylenediamine (30 µg/plate) to tannic acid, propyl gallate, ellagic acid, or gallic acid were not observed in an Ames test.*

CARCINOGENICITY STUDIES

In a study performed by the National Cancer Institute, mice were fed diets containing up to 4400 ppm 2-Nitro-p-Phenylenediamine for 78 wk.[2] *The researchers reported the administration of 2-Nitro-p-Phenylenediamine was associated with a significant dose-related increase in the combined incidence of hepatocellular adenoma and hepatocellular carcinoma in female mice, and concluded that 2-Nitro-p-Phenylenediamine was carcinogenic to female mice. Two pathologists performed independent blind evaluations of the slides of the mouse hepatic tumors and stated that a carcinogenic effect was not demonstrated in the study. Male and female rats were fed diets containing up to 1100 and 2200 ppm 2-Nitro-p-Phenylenediamine, respectively, for 78 wk. Although there were significant positive associations between 2-Nitro-p-Phenylenediamine dosage and some types of cancer, there was no convincing evidence for carcinogenicity of 2-Nitro-p-Phenylenediamine in rats.*

The International Agency for Research on Cancer (IARC) determined there is inadequate evidence in humans and limited evidence in experimental animals for the carcinogenicity of 2-Nitro-*p*-Phenylenediamine.[17] Overall, 2-Nitro-*p*-Phenylenediamine is not classifiable as to its carcinogenicity to humans (Group 3).

In Vitro Cell Transformation Assay

2-Nitro-p-Phenylenediamine (up to "100 mOSM") was studied in a BALB/c-3T3 cell transformation assay in 3 experimental trials.[3] *The test material had a sufficiently positive transformation response in the first trial and a sufficiently negative transformation response in the second trial. A third trial had sufficiently positive results and 2-Nitro-p-Phenylendiamine was determined to be "active" in this transformation assay.*

DERMAL IRRITATION AND SENSITIZATION STUDIES

In a primary skin irritation study in rabbits, 2-Nitro-p-Phenylenediamine at 2.5% was not irritating (primary irritation index (PII) = 0).[2] *2-Nitro-p-Phenylenediamine resulted in the sensitization of 4 out of 20 guinea pigs when induced and challenged at 3%.*

OCULAR IRRITATION STUDIES

2-Nitro-p-Phenylenediamine at 2.5% was slightly irritating to the rabbit eye.[2]

CLINICAL STUDIES

Retrospective and Multicenter Studies

Thirty-nine hairdressers were patch-tested with 2-Nitro-p-Phenylenediamine.[2] *Seven had previously had strong reactions to p-phenylenediamine. One of the seven was positive for 2-Nitro-p-Phenylenediamine while the other 38 hairdressers did not react to this hair dye ingredient.*

Multicenter studies of hairdressers in Europe reported sensitization rates for 2-Nitro-p-Phenylenediamine to be 4% in one study (n = 809) and 7.9% in another study (n = 302).[3] *In hairdresser clients, the sensitization rate was about 7.7% (n = 104). A retrospective European survey of allergic reactions to cosmetics in 475 patients reported 8 reactions to 2-Nitro-p-Phenylenediamine. Cross-reactions were not observed in hairdressers with known allergy to p-phenylenediamine and/or toluene-2,5-diamine sulfate.*

Case Reports

A dental hygienist who had developed dermatitis where her skin came in contact with the hair of patients was patch-tested with p-phenylenediamine and 2% 2-Nitro-p-Phenylenediamine; she had a positive reaction only for 2-Nitro-p-Phenylenediamine.[2] *Allergic contact dermatitis was observed in a 32-yr-old cleaner at a chemical plant following accidental exposure to methylenedianiline.*[3] *A patch test yielded positive results (+++) to 2-Nitro-p-Phenylenediamine at 48 and 72 h.*

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. 2-Nitro-*p*-Phenylenediamine is reported to be used in both oxidative and direct 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

2-Nitro-*p*-Phenylenediamine was previously reviewed by the Panel as part of a safety assessment with 4-Nitro-*o*-Phenylenediamine, as published in 1985. The Panel first considered a re-review of this report in 2003 and reaffirmed the 1985 conclusion, as published in 2006. In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports every 15 years, and as it had been at least 15 years since the previous re-review was issued, the Panel again considered a re-review of these ingredients at the December 2024 meeting. At that meeting, the Panel determined that this safety assessment should be re-opened for re-evaluation due to 2-Nitro-*p*-Phenylenediamine being banned for use in cosmetics by the European Union. Furthermore, the Panel determined that data for these nitro phenylenediamine hair dye ingredients could not be read-across; accordingly, rather than presenting 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine together in one amended report, re-reviews of each hair dye have been developed as individual stand-alone reports. According to the *Dictionary*, 2-Nitro-*p*-Phenylenediamine functions as a hair colorant in cosmetics. This ingredient is used in semi-permanent and permanent hair dye formulations.

According to RLD obtained from the FDA in 2025, 2-Nitro-*p*-Phenylenediamine is reported to be used in 4 hair dyes and colors. No concentrations of use were reported in the Council's 2025 survey.

European regulations regarding cosmetic ingredients categorize 2-Nitro-*p*-Phenylenediamine in Annex II, the list of substances prohibited in cosmetic products.

In a study evaluating different in vitro modeling setups using EpiSkin™ reconstructed skin, 1 mM 2-Nitro-*p*-Phenylenediamine was reported to have one of the highest penetration rates of the 6 chemicals tested. No further details about the rates were provided.

In a 20-mo dermal study, groups of male and female mice received topical applications of 0.05 ml/kg of a semi-permanent hair dye formulation containing 0.85% 2-Nitro-*p*-Phenylenediamine 3 times/wk. All mice survived until study end. Incidences of liver hemangiomas, lung adenomas, and malignant lymphomas were no greater than in the controls. No skin tumors were observed at the site of application. The NOAEL was determined to be 0.05 ml/kg. A similar study was performed in groups of male and female rats with 0.5 ml/kg applications of an oxidative hair dye formulation containing 1.1% 2-Nitro-*p*-Phenylenediamine 2 times/wk for 117 wk. All rats survived until study end. No significant increase in the incidence of tumors was observed, and no skin tumors were observed. The NOAEL was determined to be 0.5 ml/kg.

In genotoxicity studies, 2-Nitro-*p*-Phenylenediamine (50 - 500 μM) produced single strand DNA breaks in a dose-dependent manner ($p < 0.05$) in an alkaline comet assay using human lymphocytes.

The IARC determined there is inadequate evidence in humans and limited evidence in experimental animals for the carcinogenicity of 2-Nitro-*p*-Phenylenediamine. Overall, 2-Nitro-*p*-Phenylenediamine is not classifiable as to its carcinogenicity to humans (Group 3).

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

In accordance with its Procedures, the Panel re-evaluates the conclusions of previously-issued reports approximately every 15 years. In 1985, the Panel first published a final report on 2-Nitro-*p*-Phenylenediamine and concluded that this ingredient was safe as a hair dye ingredient. The Panel noted that this ingredient is a skin sensitizer in guinea pigs and that it had the potential for human sensitization. This conclusion was reaffirmed in a 2003 re-review of this ingredient, which was published in 2006. The Panel again considered a re-review of this ingredient at the December 2024 meeting and re-opened the report as 2-Nitro-*p*-Phenylenediamine has been banned for use in cosmetics by the European Commission. In this amended report, the Panel reviewed the safety of 2-Nitro-*p*-Phenylenediamine in accordance with the product categories and

concentrations of use identified in the Use section and Use table and concluded that the available data are insufficient for determining the safety of this ingredient under the intended conditions of use as a hair dye ingredient. The Panel noted a lack of relevant safety data and determined that the data needs from the Insufficient Data Announcement following the June 2025 Panel meeting remain unmet. In order to come to a conclusion of safety for this ingredient, the following additional data are needed:

- maximum concentration of use in hair dye formulations
- a 90-d oral repeated dose study with an NOAEL that shows a dose-response relationship

The Panel recognizes that coal tar hair dye ingredients are exempt from certain 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.

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

The Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings) notes that airbrush technology presents a potential safety concern. Although frequency and concentration of use data are now available (and in some cases mandated) for ingredients marketed for use with airbrush delivery systems in certain product categories, no data are available for consumer habits and practices thereof, product particle size, or other relevant particle data (e.g., diameter). As a result of deficiencies in these critical data needs, the data profile is incomplete, and the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Accordingly, the Panel has concluded that if this ingredient is used in airbrush formulations, the data are insufficient to support safe use when applied with such delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the available data are insufficient to make a determination of safety for 2-Nitro-*p*-Phenylenediamine under the intended conditions of use as a hair dye ingredient.

TABLES

Table 1. Chemical properties

Property		Value	Reference
Physical Form		Reddish-brown crystalline powder	2
		Dark green to black crystals	3
		Greenish to brownish powder	6
Molecular Weight (g/mol)		153.14	2
Density (g/ml @ 20 ºC)		0.769	6
Vapor Pressure (mmHg @ 25 ºC)		5.6×10^{-5}	6
Melting Point (ºC)		137	2
		137 - 140	3
		141.3	6
Boiling Point (ºC)		250	6
Water Solubility		Soluble in water	2
	(@ 25 ºC and 60 ºC)	Slightly soluble in water	3
	(mg/l @ 28.6 ºC)	960	6
Other Solubility		Soluble in ethanol	2
	(@ 25 ºC and 60 ºC)	Slightly soluble in isopropyl alcohol	3
log K_{ow}		0.53 (estimated)	6
		3.4 (measured)	3

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

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

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

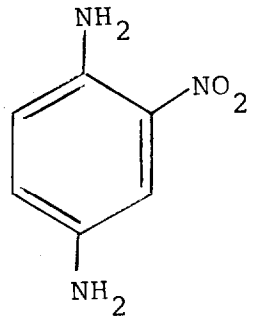
For those persons not sensitized, it is concluded that 2NPPD and 4NOPD are safe as hair dye ingredients at the current concentration of use.

INTRODUCTION

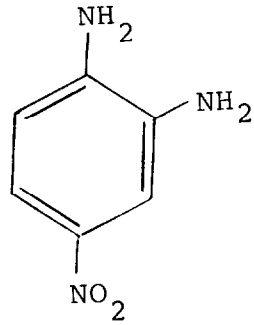
2-Nitro-*p*-phenylenediamine and 4-Nitro-*o*-phenylenediamine are reviewed in this report; they are used in both semipermanent and permanent hair dyes. *p*-Phenylenediamine is reviewed separately; it is used only in permanent hair dyes. All three compounds and other hair dye ingredients may be combined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

CHEMICAL AND PHYSICAL PROPERTIES

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



2-Nitro-*p*-phenylenediamine



4-Nitro-*o*-phenylenediamine

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

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

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

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

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

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

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

USE

Cosmetic

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

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

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

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

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

Hair-coloring formulations containing 2NPPD and 4NOPD are applied to or

may come in contact with hair, skin (particularly the scalp), eyes, and nails. Individuals dyeing their hair may use such formulations as often as once a week. Hairdressers may come in contact with products containing 2NPPD and 4NOPD several times a day.

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

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

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

Noncosmetic

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

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

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

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

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

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

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

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

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

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

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

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

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

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

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

ANIMAL TOXICOLOGY

Oral Studies

Acute Toxicity

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

Subchronic and Chronic Toxicity

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

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

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

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

Dermal Studies

Acute Toxicity

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

Subchronic Toxicity

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

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

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

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

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

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

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

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

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

Chronic Toxicity

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

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

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

Primary Skin Irritation

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

Skin Sensitization

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

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

Eye Irritation

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

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

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

TABLE 4. Eye Irritation by 2NPPD and 4NOPD

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

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

Other Studies

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

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

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

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

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

SPECIAL STUDIES

Animal Reproduction and Teratology

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

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

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

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

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

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

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

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

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

Short-Term Predictive Tests for Mutagens and Carcinogens

Mutations

Bacteria

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

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

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

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

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

TABLE 5. Mutagenicity to *Salmonella typhimurium*

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

4NOPD	0.1–1000 μg/ml, DMSO	(–)	(–)	(+)	(+)	(+)	(–)	(–)	(+)	(+)	(+)	4NOPD is also positive without S-9 in strain D3052 and is negative with and without S-9 in strains C46 and C3076	77
4NOPD	10–60 μg/plate, DMSO	–	–	(+)	(+)	(–)	–	–	–	–	–	–	78
4NOPD	0.3–333.3 μg/plate	–	–	–	(+)	–	–	–	–	(+)	–	–	79
4NOPD	–	–	(+)	(+)	–	(+)	–	–	–	–	–	–	79
4NOPD	50–100 μg/plate, DMSO	–	–	(+)	–	–	–	–	(+)	–	–	–	80
4NOPD	10 μg/plate, DMSO	–	–	–	(+)	–	–	–	–	–	–	–	81
4NOPD	50 μg/plate, DMSO	–	(+)	(+)	–	–	–	–	–	–	–	4NOPD is also positive without S-9 in strains TA97, TA90, and TA2637	82
4NOPD	–	–	–	(+)	–	–	–	–	–	–	–	4NOPD was mutagenic in TA1538 in plate tests and in liquid suspension tests only with greater than 20-hour incubations	83
4NOPD	33 nmol/ml	–	–	(+)	(+)	(+)	–	–	–	–	–	4NOPD was also positive without S-9 in strain D3052	84
4NOPD	5–50 μg/plate	–	(+)	(+)	–	–	–	–	(+)	–	–	–	97
4NOPD	10–40 μg/plate	–	(+)	(+)	(+)	(+)	–	–	–	–	–	–	86,87
4NOPD	15–150 μg/plate	–	–	–	(+)	–	–	–	–	(+)	–	4NOPD was mutagenic even after the addition of hydrogen peroxide	88
4NOPD	0.769–76.923 μg/ml	–	–	–	(+)	–	–	–	–	(+)	–	4NOPD was mutagenic even after the addition of hydrogen peroxide	89

*(+), mutagenic.
(–), nonmutagenic.

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

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

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

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

Yeast and Fungi

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

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

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

L5178Y Mouse Lymphoma Cells

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

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

Drosophila melanogaster

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

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

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

Sperm Abnormalities in Mice

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

Chromosome Damage

Human Peripheral Blood Lymphocytes

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

Chinese Hamster Cells

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

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

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

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

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

Micronucleus Test in Rats and Mice

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

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

Dominant Lethal Assay in Rats

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

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

Aneuploid Production in Fungi

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

HeLa Cell DNA Synthesis Inhibition

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

Unscheduled DNA Synthesis

HeLa Cells

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

Rat Hepatocytes

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

Morphological Transformation of Cells

Human Peripheral Blood Lymphocytes

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

C3H/10T 1/2 Mouse Cells

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

Syrian Hamster Cells

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

Rat Embryo Cells

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

Mouse Leukemia Virus Infection of Contact-Inhibited Cells

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

Carcinogenesis

Rats

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

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

Mice

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

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

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

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

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

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

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

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

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

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

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

CLINICAL ASSESSMENT OF SAFETY

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

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

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

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

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

EPIDEMIOLOGY

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

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

SUMMARY

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

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

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

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

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

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

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

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

TABLE 7. (Continued)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

DISCUSSION

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

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

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

CONCLUSION

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

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2-NITRO-*p*-PHENYLENEDIAMINE AND 4-NITRO-*o*-PHENYLENEDIAMINE

A safety assessment of 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine was published in 1985 with the conclusion "for those persons not sensitized, the Expert Panel concludes that 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine are safe as hair dye ingredients at the current concentration 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 this safety assessment.

2-Nitro-*p*-Phenylenediamine was reported to be used in 28 hair dyes and colors in 1981 at concentrations from ≤0.1% to 1% (Elder 1985). In 2002, voluntary reports provided by industry to FDA indicated that 2-Nitro-*p*-Phenylenediamine was used in 113 hair dyes and colors (FDA 2002). Use concentration data from a survey of industry practices by the Cosmetic, Toiletry, and Fragrance Association (CTFA) indicated use at concentrations from 0.1% to 1% in cosmetic products (CTFA 2003).

4-Nitro-*o*-Phenylenediamine was reported to be used in 26 hair dyes and colors in 1981, at concentrations of ≤ 0.1% to 1% (Elder 1985). Industry reports to FDA in 2002 included 22 uses as hair dyes and colors. Use concentration data from an industry survey in 2003 indicated use at concentrations of 0.1% to 0.2% (CTFA 2003).

The available use and concentration as a function of product type is given in Table 13. The most recent information now constitutes the current practices of use and concentration.

In 2003, an updated review of the available hair dye epidemiology literature was prepared (Helzlsouer et al. 2003). 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 bladder cancer, non-Hodgkin's lymphoma, and multiple myeloma. These findings, however, were not consistently observed across studies.

In considering all these 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. The Panel stated that use of direct

[redacted]

TABLE 13
Historical and current uses and use concentrations for 2-Nitro-*p*-phenylenediamine and 4-Nitro-*o*-phenylenediamine

Product category	1981 use (Elder 1980)	2002 use (FDA 2002)	1981 concentrations (Elder 1980) %	2003 concentrations (CTFA 2003) %
	2-Nitro-*p*-phenylenediamine			
Hair dyes and colors	28	113	≤0.1–1	0.1–1
Total uses/ranges for 2-Nitro-*p*-phenylenediamine	**28**	**113**	**≤0.1–1**	**0.1–1**
	4-Nitro-*o*-phenylenediamine			
Hair dyes and colors	26	22	≤0.1–1	0.1–0.2
Total uses/ranges 4-Nitro-*o*-phenylenediamine	**26**	**22**	**≤0.1–1**	**0.1–0.2**

hair dyes, although not the focus in all investigations, appears 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.

Discussion of the most recent available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml.

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OLEIC ACID, LAURIC ACID, PALMITIC ACID, MYRISTIC ACID, AND STEARIC ACID

A safety assessment of the Oleic Acid group was published in 1987 with a conclusion that these ingredients are safe in present practices of use and concentration in cosmetics. New studies regarding these fatty acids available since then, along with updated information regarding uses and use concentrations, were considered by the CIR Expert Panel. The Panel determined to not reopen this safety assessment.

Oleic Acid usage increased from 424 in 1981 to 1131 in 2002, based on industry voluntary reports provided to FDA (Elder 1987; FDA 2002). An industry survey in 2004 indicated that use concentrations range from 0.00004% to 20%, within the range reported in 1981 (Elder 1987).

Lauric Acid usage increased from 22 in 1981 to 121 in 2002, based on industry voluntary reports provided to FDA (Elder 1987; FDA 2002). An industry survey in 2004 indicated that use concentrations range from 0.00003% to 11%, within the range reported in 1981 (Elder 1987).

Palmitic Acid usage increased from 29 in 1981 to 132 in 2002, based on industry voluntary reports provided to FDA (Elder 1987; FDA 2002). An industry survey in 2004 indicated that use concentrations range from 0.00006% to 20%, within the range reported in 1981 (Elder 1987).

Myristic Acid usage increased from 36 in 1981 to 73 in 2002, based on industry voluntary reports provided to FDA (Elder 1987; FDA 2002). An industry survey in 2004 indicated that use concentrations range from 0.00001% to 38%, within the range reported in 1981 (Elder 1987).

Stearic Acid usage decreased from 2465 in 1981 to 2133 in 2002, based on industry voluntary reports provided to FDA (Elder 1987; FDA 2002). An industry survey in 2004 indicated that use concentrations range from 0.000002% to 43%, within the range reported in 1981 (Elder 1987).

The available use and concentration data are given in Table 14. The most recent information now constitutes the present practices of use and concentration.

The newly available studies reported findings consistent with the data in the original safety assessment. One area not covered in the original report was reproductive and developmental toxicity. One new study was available that demonstrated little or no toxicity to sperm cells by Oleic Acid, Palmitic Acid, and Stearic Acid.

These fatty acids may be plant derived. In such cases, established limits for pesticide and heavy metal residues should not be exceeded (lead $\leq$10 ppm, arsenic $\leq$3 ppm, mercury $\leq$1 ppm, total PCB/pesticide $\leq$40 ppm, with $\leq$10 ppm for any specific pesticide residue).

These fatty acids may also be derived from animal sources, including beef. The Panel agrees with the Food and Drug Administration's position that tallow derivatives, including these fatty acids, would not present any risk of transmissible encephalopathies.

C I R

November 14, 2003

Memorandum

To: CIR Expert Panel

From: Wilbur Johnson, Jr.
Senior Scientific Analyst

Subject: Re-review of 2-Nitro-*p*-Phenylenediamine (2NPPD) and 4-Nitro-*o*-Phenylenediamine (4NOPD)

In 1985, CIR published a Final Report with the following conclusion: 2NPPD and 4NOPD are skin sensitizers for guinea pigs. Information in this report and in the report on PPDA (p-Phenylenediamine) suggests that 2NPPD and 4NOPD have potential for human sensitization. For those persons not sensitized, the Expert Panel concludes that 2NPPD and 4NOPD are safe as hair dye ingredients at the current concentration of use. A copy of this Final Report is included.

A search of the currently available scientific literature uncovered numerous studies on 2-Nitro-p-phenylenediamine/4-Nitro-o-Phenylenediqamine. The available data are summarized in the attached re-review background document along with frequency of use data provided in 2002. The 1993 IARC report on 2-Nitro-p-Phenylenediamine is also attached for the Panel's consideration.

The task for the Panel at this meeting is to determine whether the conclusion on 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine is still valid. If it is not, an amendment should be initiated. If the conclusion is still valid, then the Panel should decide if there is a need for an addendum to add significant new safety or other data. If there is no such need, the Panel may simply describe the new information considered and reaffirm the original conclusion.

RE-REVIEW DOCUMENT ON 2-NITRO-*p*-PHENYLENEDIAMINE (2NPPD) AND 4-NITRO-*o*-PHENYLENEDIAMINE (4NOPD)

The two aromatic amines, 2-Nitro-p-Phenylenediamine and 2-Nitro-p-Phenylenediamine, are listed in the *International Cosmetic Ingredient Dictionary and Handbook* (Pepe et al., 2002). The CIR Expert Panel has evaluated the safety of these two Phenylenediamines in cosmetics, and a Final Report with the following conclusion was published in 1985: 2NPPD and 4NOPD are skin sensitizers for guinea pigs. Information in this report and in the report on PPDA (*p*-Phenylenediamine) suggests that 2NPPD and 4NOPD have potential for human sensitization. For those persons not sensitized, the Expert Panel concludes that 2NPPD and 4NOPD are safe as hair dye ingredients at the current concentration of use (Elder, 1985).

An updated search of the literature was performed to identify studies on 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine that have been published since the Panel's Final Safety Assessment was issued. These studies, summarized in text, will be used to determine whether reevaluation of the safety of these ingredients in cosmetics by the Panel is warranted.

CHEMISTRY

DEFINITION AND PROPERTIES

2-Nitro-*p*-Phenylenediamine

According to Pepe et al. (2002), the substituted aromatic amine 2-Nitro-*p*-Phenylenediamine (CAS No. 5307-14-2) is a hair colorant. Other technical names for

this chemical include: 4-Amino-2-nitroaniline, 1,4-Benzenediamine, 2-Nitro-, CI 76070, 2,5-Diaminonitrobenzene, 2-Nitro-1,4-Diaminobenzene, o-Nitro-p-phenylenediamine, and Oxidation Base 9A.

2-Nitro-*p*-Phenylenediamine has a molecular weight of 153.14 and is slightly soluble in water (at 25°C and 60°C) and in isopropyl alcohol (at 25°C and 60°C). The product specification for 2-Nitro-*p*-Phenylenediamine is as follows: dark green to black crystals (description), 98.0% min (purity), 0.1% max (ash), 137 to 140°C (melting point), 50 ppm max (Iron), and infrared spectrum (conforms to standard) (Keystone Aniline Corporation,1999).

4-Nitro-*o*-Phenylenediamine

According to Pepe et al. (2002), the substituted aromatic amine 4-Nitro-o-Phenylenediamine (CAS No. 99-56-9) is a hair colorant. Other technical names for this chemical include: 2-Amino-4-nitroaniline; 1,2-Benzenediamine, 4-Nitro-; 3,4-Diaminonitrobenzene, and 4-Nitro-1,2-Diaminobenzene.

4-Nitro-*o*-Phenylenediamine has a molecular weight of 153.14 and is slightly soluble in water (at 25°C and 60°C) and in isopropyl alcohol (at 25°C and 60°C). The product specification for 4-Nitro-*o*-Phenylenediamine is as follows: red to orange brown powder (description), 99.5% min (purity), 0.1% max (ash), 92 to 95°C (melting point), 40 ppm max (Iron), infrared spectrum (conforms to standard) (Keystone Aniline Corporation,1999).

REACTIVITY

According to Crank and Makin (1984), superoxide ion acts as a mild and highly

selective oxidizing agent for aromatic amines. O- and p-diamines are oxidized to diaminoazobenzenes. The oxidations are considered free-radical processes, initiated by hydrogen abstraction by superoxide from the substrates.

USE

PURPOSE IN COSMETICS

4-Nitro-o-Phenylenediamine and 2-Nitro-*p*-Phenylenediamine function as hair colorants in cosmetic products (Pepe et al., 2002).

According to Keystone Aniline Corporation (1999), 2-Nitro-*p*-Phenylenediamine and 4-Nitro-*o*-Phenylenediamine are categorized as semipermanent dye and oxidation dye intermediates, and their primary use is that of a direct colorant and/or secondary intermediate.

SCOPE AND EXTENT OF USE IN COSMETICS

Current frequency of use data provided by FDA in 2002 are summarized in Table 1 along with similar data from the CIR Final Report that was published in 1985. Current use concentration data, from a cosmetics industry survey, are anticipated (CTFA, 2003).

INTERNATIONAL USE

Though 2-Nitro-*p*-Phenylenediamine is not included on this list, it may be important to note that Nitro-*p*-Phenylenediamine and its salts are included on the list of ingredients of quasi drugs that are marketed in Japan. Some cosmetic products are considered quasi-drugs. For example, in Japan, products used to improve such symptoms as chapped skin, prickly heat, sores, corns, calluses, and dry skin are among

Table 1. Product Formulation Data on 2-Nitro-*p*-phenylenediamine and 4-Nitro-*o*-phenylenediamine

Product Category (Number of Formulations Reported to FDA) (FDA, 2002)		Number of Formulations Containing Ingredient (Elder, 1980)	Number of Formulations Containing Ingredient (FDA, 2002)	Concentration of Use (Elder, 1980)	Concentration of Use (CTFA, 2003)
2-Nitro-*p*-phenylenediamine					
Hair Dyes and Colors (1690)		28	113	≤ 0.1 to 1%	0.1 to 1%
	Totals	28	113		
4-Nitro-*o*-phenylenediamine					
Hair Dyes and Colors (1690)		26	22	≤ 0.1 to 1%	0.1 to 0.2%
	Totals	26	22		

those classified as quasi-drugs (Ministry of Health, Labor and Welfare [MHLW], 2001).

2-Nitro-*p*-Phenylenediamine and its salts are permitted for use as both oxidizing and non-oxidizing coloring agents for hair dyeing in cosmetic products marketed within the European Union. For both product types (oxidizing and non-oxidizing hair dyes), the maximum authorized concentration for 2-Nitro-p-Phenylenediamine and its salts is 0.3%. Additionally, in combination with hydrogen peroxide, the maximum use concentration upon application is 0.15% (European Economic Community, 1999).

BIOLOGICAL PROPERTIES

ABSORPTION AND METABOLISM

4-Nitro-o-Phenylenediamine and 2-Nitro-p-Phenylenediamine

Wolfram and Maibach (1985) studied the percutaneous penetration of hair dyes in three monkeys and three human subjects. The scalp penetration of seven hair dyes (oxidative and direct) that occurs under conditions of hair dye usage was evaluated using ^{14}C-labeled materials by quantifying their absorption via urine assays. The assay results refer mostly to data obtained during the 144-hour period following application of the dye. Both species showed a remarkably similar pattern of dye penetration. For

human subjects and monkeys values for the total dose excretion (%) of 2-Nitro-p-Phenylenediamine were 0.143 ± 0.04 and 0.551 ± 0.10,respectively. The half time ($T_{1/2}$) of urinary excretion of 2-Nitro-p-Phenylenediamine was 24 hours.

Nakao et al. (1987) studied the metabolism of 2-Nitro-p-Phenylenediamine using male Sprague-Dawley rats (6 weeks old; weight = 200g). The rats were dosed intraperitoneally with the test substance (dissolved in a 2% carboxymethyl cellulose sodium salt solution; dose = 100 mg/5ml/kg). Urine was collected for 24 hours. Two major metabolites, N^4-acetyl-2-nitro-1,4-diaminobenzene and N^1,N^4-diacetyl-1,2,4-triaminobenzene were isolated and identified.

In a study by Nakao et al. (1991), reductive metabolism of 2-Nitro-p-Phenylenediamine and its acetylated metabolite, 2-Nitro-p-Phenylenediamine N^4-acetate was investigated with rat liver subcellular fractions, microsomes, and cytosol (from male Sprague-Dawley rats weighing 200 to 215 g). Under aerobic conditions, these compounds were reduced to their corresponding amines by these fractions.

In a study by Bronaugh and Congdon (1984), the correlation between the percutaneous absorption of hair dyes and their partition coefficients was evaluated. The absorption of hair dyes through human epidermis (abdominal skin) was measured using a diffusion cell. Octanol/water partition coefficients were determined by shaking the test compound in a mixture containing water and octanol. The ratio of the amount of compound in each solvent was determined. The octanol/water partition coefficient for 2-Nitro-p-Phenylenediamine was 3.4.

The absorption of hair dyes through excised human skin was observed when they were applied in aqueous vehicle. Permeation was reduced under conditions that

resulted in ionization of a compound. The authors stated that the relationship of partition coefficients to permeability measurements is somewhat tenuous, and depends on the compounds in question. Since other factors such as binding to proteins in the skin play a role in determining absorption rate, oil/water partition coefficients, by themselves, gave limited predictive information (Bronaugh and Congdon, 1984).

The percutaneous absorption of 2-Nitro-p-Phenylenediamine and other dyes was measured through human and monkey skin in a study by Bronaugh and Maibach (1985). Rapid penetration of the test compound was observed, with maximum absorption occurring during the first few hours. A comparison of the human and monkey *in vitro* data showed a trend toward increased absorption through monkey skin.

Yourick and Bronaugh (2000) initiated *in vitro* studies to measure absorption and metabolism of 2-Nitro-p-Phenylenediamine in human and fuzzy rat skin and rat jejunal tissue. Absorption was measured over 24 hours by using flow-through diffusion cells. Dosing vehicles were applied to the skin and intestine in the diffusion cells for 30 minutes. Metabolites were determined using high-performance liquid chromatography. Female Fuzzy (Hsd:Fuzzy-fz) rats (3 to 10 months old) were used. Fresh, viable human skin was obtained as a result of abdominoplasty procedures from a local cosmetic surgeon. The results suggest that 2-Nitro-p-Phenylenediamine is rapidly absorbed and extensively metabolized in both skin and intestinal tissue. In human and rat skin, it was metabolized to triaminobenzene and N4-acetyl-2-Nitro-p-Phenylenediamine. 2-Nitro-p-Phenylenediamine was also metabolized to a sulfated metabolite in rat skin, but not in human skin.

TOXICOLOGY

MUTAGENICITY/GENOTOXICITY

Ames Test

4-Nitro-o-Phenylenediamine and 2-Nitro-p-Phenylenediamine

In a study by Dunkel et al. (1985), both chemicals were evaluated in the Ames test using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and *E. coli* strain WP2. The tests were conducted at four different laboratories, and the highest doses tested did not exceed 6,666 µg/plate (2-Nitro-p-Phenylenediamine) or 10,000 µg/plate (4-Nitro-o-Phenylenediamine) at either of the laboratories. Overall test results indicated that these two chemicals were judged mutagenic in the same strain/activation combination (at least one) in all laboratories.

Kvelland (1985) evaluated the mutagenicity of 4-Nitro-o-Phenylenediamine (dissolved in dimethyl sulfoxide and diluted with ethanol) using Bacteriophage T4D (E. coli strains B, CR63, and K12 (λh bacterial strains employed). The induction of rapid lysis mutants in bacteriophage T4D was used as a tool for measuring any possible mutagenic effect. Test substance concentrations in the assay ranged from 7.4 to 521.3 µg/ml. Only one dose of 4-Nitro-o-Phenylenediamine (384.6 µg/ml) showed a weak mutagenic effect (significantly higher than the control at the 5% level).

In a study by Popkin and Prival (1985), the mutagenic activity of 4-Nitro-o-Phenylenediamine was affected by pH. In *Salmonella typhimurium* strain TA1537, the test substance (dose = 10 mg/plate, without metabolic activation) had an optimal mutagenic response at pH 6.3. At the base agar pH normally used in the Ames test

(pH 7.0), the response was only approximately 0.6 of that observed at optimal pH. In strain TA100, the test substance (dose = 10 µg/plate) had an optimal mutagenic response at pH 6.5.

In a study by Lee et al. (1988), 4-Nitro-o-Phenylenediamine and 2-Nitro-p-Phenylenediamine were each mutagenic to *Salmonella typhimurium* strain TA98 with and without metabolic activation. 4-Nitro-o-Phenylenediamine was the more potent mutagen in the absence of metabolic activation, and the opposite was true in the presence of metabolic activation. For both chemicals, the number of revertants per plate increased with increasing doses (0 to 100 µg/plate) both with and without metabolic activation.

In another test (Lee et al., 1986) using *Salmonella typhimurium* strain TA 98, the numbers of revertants per plate at the highest test concentration of 4-Nitro-o-Phenylenediamine (100 µg/plate) were 7112 without metabolic activation and 1126 with metabolic activation. The numbers of revertants per plate at the highest test concentration of 2-Nitro-p-Phenylenediamine (100 µg/plate) were 251 without metabolic activation and 3688 with metabolic activation. Values for control cultures were 16 (without metabolic activation) and 28 (with metabolic activation).

In a study by Chung et al. (1995), 2-Nitro-p-Phenylenediamine (doses up to 3,000 µg/plate) was mutagenic to *Salmonella typhimurium* strains TA98 and TA100 with and without metabolic activation.

In a study by Chung et al. (1996), both 4-Nitro-o-Phenylenediamine and 2-Nitro-p-Phenylenediamine (doses up to 3,000 µg/plate) were mutagenic to the following *Salmonella typhimurium* strains with and without metabolic activation: TA98, TA100,

TA98NR, and TA100NR.

In the Ames test, the mutagenicity of 4-Nitro-o-Phenylenediamine and 2-Nitro-p-Phenylenediamine toward *Salmonella typhimurium* strains TA100, TA104, TA4001, and TA4006 was evaluated. 4-Nitro-o-Phenylenediamine was tested at doses up to 300 µg/plate and 2-Nitro-p-Phenylenediamine was tested at doses up to 1000 µg/plate. Both compounds were mutagenic (dose-response) to strains TA100 and 104, but were not mutagenic to strain 4001. 2-Nitro-p-Phenylenediamine was weakly mutagenic toward strain TA4006, and the response was similar for 4-Nitro-o-Phenylenediamine (Chen et al., 1997).

In the Ames test without metabolic activation, 4-Nitro-o-Phenylenediamine (dose = 10 µg/plate) and 2-Nitro-p-Phenylenediamine (dose = 30 µg/plate) were mutagenic to *Salmonella typhimurium* strain TA98 (Chen and Chung, 2000).

The following *Salmonella typhimurium* strains were used to examine the mutational specificity of 4-Nitro-o-Phenylenediamine and 2-Nitro-p-Phenylenediamine, and other aromatic amines that have been shown to cause missense mutations in *Salmonella* tester strain TA100: TA7001, TA7002, TA7003, TA7004, TA7005, and TA7006. Results suggest that 4-Nitro-o-Phenylenediamine and 2-Nitro-p-Phenylenediamine induced TA → AT and CG → AT transversions as well as GC → AT transitions in the *Salmonella typhimurium his* gene. 2-Nitro-p-Phenylenediamine also induced a small portion of CG → GC transversions (Chung et al., 2000).

Furthermore, these results demonstrate that CG →TA transitions and CG →AT transversions are the major types of mutation induced by nitro-containing aromatic amines in TA100 (Chung et al., 2000).

L5178Y Mouse Lymphoma Assay

4-Nitro-o-Phenylenediamine

In a study by Oberly et al. (1984), 4-Nitro-o-Phenylenediamine was tested at doses up to 200 µg/ml in the L5178Y mouse lymphoma assay. A positive response for chemical-induced $TK^{-/-}$ mutation was obtained without metabolic activation. The positive response was demonstrated by a dose-related increase in mutation frequency.

In a study by Myhr and Caspary (1988), an assay conducted according to the method of Clive and Spector (1975). Dose-related increases in mutant frequency were reported in three trials under nonactivation conditions. Responses began near 75 to 150 µg/ml and reached maximum values of approximately three- to sixfold increases in mutant frequency near the solubility limit (375 to 450 µg/ml).

In the presence of metabolic activation, the compound became more toxic and induced significant increases in mutant frequency at concentrations as low as 20 to 30 µg/ml. The first activation trial showed only a 2l.3-fold increase in mutant frequency for the high dose of 100 µg/ml, which was very toxic (10% relative total growth). Lower concentrations with less toxicity were effective in inducing mutagenic responses in the next two experiments. The largest response (fourfold) was obtained at 150 µg/ml in trial 3 and was equivalent to the response obtained under nonactivation conditions at 150 µg/ml in trial 2 (Myhr and Caspary, 1988).

In a study by Mitchell et al. (1988), the mouse lymphoma assay was conducted according to the method of Clive and Spector (1975). Mutagenic responses were observed with and without metabolic activation. In the first experiment without activation, the mutant frequency was increased 2.3-fold (14% relative total growth) at

256 µg/ml. In the second trial, an approximately threefold mutagenic response (24% relative total growth) was observed at 228 µg/ml.

Mutagenicity was increased in the presence of metabolic activation, with an approximately threefold increase observed at 50 µg/ml in the first trial. In the second trial, a fivefold increase was observed at 40 µg/ml and 50 µg/ml was toxic. Thus, the mutagenicity of 4-Nitro-o-Phenylenediamine was increased and cytotoxicity was observed at lower concentrations following exposures in the presence of metabolic activation than in its absence (Mitchell et al., 1988).

2-Nitro-p-Phenylenediamine

In a study by Oberly et al. (1984), 2-Nitro-p-Phenylenediamine was tested at doses up to 400 µg/ml in the L5178Y mouse lymphoma assay. A positive response for chemical-induced $TK^{-/-}$ mutation was obtained without metabolic activation. The positive response was demonstrated by a dose-related increase in mutation frequency.

In a study by Mitchell et al. (1988), the mouse lymphoma assay conducted according to method of Clive and Spector (1975). Cytotoxic and mutagenic responses were noted at doses above 130 µg/ml in the absence of metabolic activation. Mutant frequencies were increased approximately fivefold at 450 µg/ml in one trial and at 500 µg/ml in the other.

Mutagenic effects in the presence of metabolic activation were difficult to evaluate. The chemical was cytotoxic at much lower concentrations (1 to approximately 10 µg/ml). In the first experiment, an increased mutant frequency was observed, but was not statistically significant, even though the relative total growth was less than 10%. In the second trial, the mutant frequency was significantly increased in cultures exposed

to 4.9, 7.7, and 12 µg/ml (8% relative total growth). In spite of the lack of a significant response in the first trial, the observed response at 6 µg/ml was consistent with the response in the second experiment. Because the trend in the first experiment supported the positive response in the second, the test with metabolic activation was evaluated as positive (Mitchell et al., 1988).

Chromosomal Aberrations

4-Nitro-o-Phenylenediamine

In a study by Lee et al. (1986), 4-Nitro-o-Phenylenediamine induced a significant increase in the number of sister chromatid exchanges in Chinese hamster ovary cells both with and without metabolic activation. At the highest concentration tested (1×10^{-3} M), the mean number of sister chromatid exchanges per cell was 10.3 ± 0.23 without metabolic activation, and 13.4 ± 0.30 with metabolic activation. In control cultures, mean numbers of sister chromatid exchanges per cell were 5.4 ± 0.13 without metabolic activation and 7.3 ± 0.20 with metabolic activation.

McFee et al. (1989) studied the induction of chromosomal aberrations in mouse bone marrow cells (from male B6C3F1 mice; body weight = 25 to 30 g). The test substance was injected intraperitoneally at doses up to 1,000 mg/kg. The number of aberrations observed after dosing with 4-Nitro-o-Phenylenediamine was slightly below the control number and showed no suggestion of an effect of the compound. The micronucleus frequency was slightly higher among mice dosed with the test substance than in the controls; however, a statistically significant response was observed only at the 24-hour sampling time. An increase in the number of sister chromatid exchanges was noted after dosing with 4-Nitro-o-Phenylenediamine; however, the trend test

analysis did not indicate a statistically significant response.

In a study by Soler-Niedziela et al. (1991), the mutagenicity of 4-Nitro-o-Phenylenediamine was evaluated in the micronucleus test. Groups of five male CD-1 mice (weights = 23 to 30 g) were injected intraperitoneally with the test substance (doses up to 500 mg/kg). The animals were killed by cervical dislocation and bone marrow smears prepared. Results indicated that mice dosed with 4-Nitro-o-Phenylenediamine did not exhibit any dose-related response in micronuclei induction over the three dose levels tested. Ratios of polychromatic erythrocytes to normochromatic erythrocytes were not significantly different from the solvent control.

In a study by Neal and Probst (1983), 4-Nitro-o-Phenylenediamine, administered orally to three inbred female Chinese hamsters (*Cricetulus griseus*, weights = 26 to 32 g), did not increase bone marrow-sister chromatid exchange frequency. The test substance was administered orally at doses up to 500 mg/kg.

In a study by Neal and Probst (1984), sister chromatid exchange was investigated in differentiating spermatogonia of the Chinese hamster as a model for detecting genotoxins that were capable of interacting with germinal tissue. The induction of sister chromatid exchanges in spermatogonia and bone marrow were compared. The induction of sister chromatid exchanges in intestinal epithelium was also examined. Both 2-Nitro-p-Phenylenediamine and 4-Nitro-o-Phenylenediamine were tested. Oral administration of either chemical (suspensions in 10% aqueous acacia) failed to induce sister chromatid exchanges in bone marrow. 2-Nitro-p-Phenylenediamine induced a dose-related increase (oral doses up to 500 mg/kg) increase in sister chromatid exchanges in intestinal epithelium. This response was not

evident in animals dosed orally with 4-Nitro-p-Phenylenediamine.

2-Nitro-p-Phenylenediamine

In a study by Neal and Probst (1983), the mutagenicity of 2-Nitro-p-Phenylenediamine was evaluated in the *in vivo* sister chromatid exchange assay. Results were negative when the test substance was administered orally (doses up to 500 mg/kg) or intraperitoneally (doses up to 300 mg/kg). Groups of three inbred female Chinese hamsters (*Cricetulus griseus*) were used.

Adam (1985) evaluated the mutagenicity of 2-Nitro-p-Phenylenediamine. The chemical was administered intraperitoneally to male CFW inbred mice at doses of 0.2 LD50 and 0.5 LD50. Chromosomal aberrations in metaphases of bone marrow and Ehrlich ascites tumor cells were estimated. The test substance did not cause significant and repetitive changes in mitotic frequency in bone marrow and ascites tumor cells.

In a study by Lee et al. (1986), 2-Nitro-p-Phenylenediamine induced a significant increase in the number of sister chromatid exchanges in Chinese hamster ovary cells both with and without metabolic activation. At the highest concentration tested (1×10^{-3} M), the mean number of sister chromatid exchanges per cell was 13.9 ± 0.34 without metabolic activation, and 12.9 ± 0.52 with metabolic activation. In control cultures, mean numbers of sister chromatid exchanges per cell were 5.4 ± 0.13 without metabolic activation and 7.3 ± 0.20 with metabolic activation.

In a study by Misra (1992), the micronucleus test was used to evaluate the genotoxicity of 2-Nitro-p-Phenylenediamine. Groups of ten male Swiss mice (8 weeks old; weight maintained at approximately 30 g) were administered intraperitoneal

injections of the test substance at doses of 50, 100, 200, 250, 500, and 1000 mg/kg body weight, respectively, twice (24-hour interval between the 2 doses). The animals were killed six hours after the second injection, and bone marrow smears prepared. Death was observed at the highest dose administered, and cytotoxicity was observed at all doses. 2-Nitro-p-Phenylenediamine induced a dose-related increase in micronuclei in polychromatic and normochromatic bone marrow cells. The results were statistically significant ($p < 0.02$). 2-Nitro-p-Phenylenediamine was clastogenic.

Chung et al. (1995) evaluated the potential for 2-Nitro-p-Phenylenediamine (doses up to 738 µg/ml) to induce chromosomal aberrations without metabolic activation using Chinese hamster ovary cells (CHO-K1). 2-Nitro-p-Phenylenediamine was evaluated at doses that were equivalent to ½ x, 1 x, 2 x, and 3 x the TC50 (50% toxic concentration). The TC50 for 2-Nitro-p-Phenylenedimine was 246 ± 24 µg/ml. A positive dose-related increase in chromosomal aberrations was observed. Gaps, breaks, deletions, exchanges, and dicentrics were present.

Alkaline Single Cell Gel Electrophoresis Assay

4-Nitro-o-Phenylenediamine

Sasaki et al. (1999) evaluated the genotoxicity of 4-Nitro-o-Phenylenediamine using the alkaline single cell gel electrophoresis (SCG) assay. Four male ddy mice (6 weeks old) were dosed orally (gavage) with 500 mg/kg. The animals were killed 0, 3, 8, and 24 hours after dosing and eight organs (stomach, colon, liver, kidney, urinary bladder, lung, brain, and bone marrow) taken and subjected to necropsy and microscopic examination. Slides for the SCG assay were prepared using nuclei isolated by homogenization. Data on the migration of nuclear DNA from organs

examined were presented. 4-Nitro-o-Phenylenediamine did not yield a statistically significant increase in DNA damage in any mouse organ examined that was studied.

E.coli K-12 *uvr*B/*rec*A DNA Repair Host-Mediated Assay

4-Nitro-o-Phenylenediamine and 2-Nitro-p-Phenylenediamine

In a study by Hellmér and Bolcsfoldi (1992), the genotoxicity of 4-Nitro-o-Phenylenediamine and 2-Nitro-p-Phenylenediamine was evaluated in the E. coli K-12 *uvr*B/*rec*A DNA repair host-mediated assay. The assay involves the administration of the test substance to mice by the route of choice, followed by intravenous administration of a mixture of DNA repair deficient and proficient derivatives of E. coli K-12. After an incubation period, the relative survival of the two strains was determined in blood, liver, lungs, kidneys, and testes of the host. A significant preferential reduction of the DNA repair deficient strain in any organ indicates that the test substance possesses genotoxic properties.

Two groups of male NMRI mice were dosed orally with 230 mg/kg and 700 mg/kg 4-Nitro-o-Phenylenediamine, respectively. Three groups of mice were dosed orally with 160 mg/kg, 330 mg/kg, and 660 mg/kg 2-Nitro-p-Phenylenediamine, respectively. Assay results were positive for 2-Nitro-p-Phenylenediamine (blood, lungs, and kidneys), but negative for 4-Nitro-o-Phenylenediamine (Hellmér and Bolcsfoldi, 1992).

Unscheduled DNA Synthesis

4-Nitro-o-Phenylenediamine

van Erp et al. (1992) investigated unscheduled DNA synthesis in the human hair

follicle after exposure to 4-Nitro-o-Phenylenediamine *in vitro*. Hair follicles were exposed to the test substance at concentrations of 3 to 10 µg/ml. Results were negative without metabolic activation. The experiment was not conducted with metabolic activation.

In a study by Williams (1997), the genotoxicity of 4-Nitro-o-Phenylenediamine (test concentration not stated) was evaluated according to the rat hepatocyte/unscheduled DNA synthesis repair test procedure by Williams et al. (1982). Results were negative.

Cell Transformation Assay

4-Nitro-o-Phenylenediamine

In a study by Matthews et al. (1993), the genotoxicity of 4-Nitro-o-Phenylenediamine was evaluated in the cell transformation assay (BALB/c-3T3 cells). The upper dose limit was 100 mOsM. Transformed foci of BALB/c-3T3 cells were identified microscopically. The test substance had a sufficiently positive transformation response in the first experiment and a limited activity transformation response in the second experiment. 4-Nitro-o-Phenylenediamine was evaluated as active in this assay.

The ability of the pH 6.7 Syrian hamster embryo cell transformation assay to detect accurately chemicals of known carcinogenic activity, as defined in rodent cancer models, was determined in a study by LeBoeuf et al. (1996). The maximum test concentration for 4-Nitro-o-Phenylenediamine was the concentration that was expected to result in (a) at least a 50% reduction in the relative plating efficiency, compared to the solvent control; (b) the concentration that was visibly insoluble in the culture medium and resulted in no further decrease in relative plating efficiency; or (c) a maximum of 5

mg/ml in the assay. Both a 24-hour and a 7-day exposure procedure was used. At the end of the culture period, the cultures were rinsed, fixed, and stained. The cultures were then scored for total colony number and identification of morphologically transformed colonies. Morphologically transformed colonies contain cells in an extensive random-oriented, three dimensional, stacked growth pattern, with criss-crossing cells at the perimeter and in the center of the colony. Results for 4-Nitro-o-Phenylenediamine were negative.

In another study (Kerckaert et al., 1998), 4-Nitro-o-Phenylenediamine did not yield significant morphological transformation at any of the doses tested (up to ~300 mg/ml) in the pH 6.7 Syrian hamster embryo cell transformation assay.

2-Nitro-p-Phenylenediamine

In a study by Matthews et al. (1993), the genotoxicity of 2-Nitro-p-Phenylenediamine was evaluated in the cell transformation assay (BALB/c-3T3 cells). The upper dose limit was 100 mOsM. Transformed foci of BALB/c-3T3 cells were identified microscopically. In the first experimental trial, 2-Nitro-p-Phenylenediamine had a sufficiently positive transformation response. In experimental trial 2, the chemical had a sufficiently negative transformation response. Based on these results, a third experimental trial was conducted, and the results were sufficiently positive. 2-Nitro-p-Phenylenediamine was evaluated as active in the transformation assay.

Transgenic Mouse Mutation Assay

2-Nitro-p-Phenylenediamine

In a study by Suter et al. (1996), the induction of mutations in the *lac*I gene in the

liver of transgenic Big Blue C57BL/6 mice was evaluated. Doses of 150 mg/kg/day were administered daily for 10 days over a two-week period and the animals were killed. Compared to vehicle controls (0.5% hydroxypropyl methylcelluslose), 2-Nitro-p-Phenylenediamine increased the mutant frequency in males by a factor of 2. There was no increase in females.

Drosophila Short-Term Somatic Assays

Some of the assays involving *Drosophila melanogaster* conducted below are explained as follows (Vogel et al., 1999): The genetic principle of the white-ivory system is somatic reversion of the X-chromosomal, recessive eye color mutation, *white-ivory* (w^i) to wild type (w^+). Reversions result in red (w^+) clones of otherwise white-ivory eyes.

The zeste-white assay is based on phenotypically visible changes in eye pigmentation, for which the activity of the white locus is responsible. Unstable zeste-white strains contain a white locus that is unstable due to insertion of a piece of DNA in the locus (Vogel et al., 1999).

4-Nitro-o-Phenylenediamine

Batiste-Alentorn et al. (1995) evaluated the mutagenicity of 4-Nitro-o-Phenylenediamine in three short-term somatic mutation assays. To determine the induction of somatic mutations in the zeste-white test, the emerging males (*Drosophila melanogaster*) from the different treatments were examined, in both eyes, for eye color mosaicism. Red (or white) spots consisting of 4 or more ommatidia were scored. Males with both eyes red (or white) were presumed to be due to germ-line mutations or deletions and were not recorded.

To determine the induction of somatic mutations in the white ivory test, emerging males from the different treatments were examined for eye color mosaicism (in both eyes). The number of pigmented ommatidia was scored. The effect of the treatments on the developing eye was calculated as the frequency of sectors with one or more ommatidia with altered pigmentation among the scored eyes (Batiste-Alentorn et al., 1995).

In the wing spot test, the wings were removed, mounted, and scored for the presence of clones of cells showing malformed wing hairs (Batiste-Alentorn et al., 1995).

4-Nitro-o-Phenylenediamine induced a significant increase in the frequency of eye-color mosaicism in the zeste-white test at the highest concentration assayed (2 mM). Inconclusive results were produced in the white ivory test and the wing spot test. 4-Nitro-o-Phenylenediamine produced significant increases in the frequency of mutant clones in at least one of the somatic assays. It was concluded that the wing spot test was the most effective assay in terms of detecting genotoxicity (Batiste-Alentorn et al., 1995).

Rodriguez-Arnaiz and Aranda (1994) evaluated the mutagenicity of 4-Nitro-o-Phenylenediamine in the w/w$^+$ somatic assay using *Drosophila melanogaster*. This assay monitors a diverse set of DNA lesions in somatic cells of treated *Drosophila* larvae. Data obtained for somatic eye mutations and mitotic recombination using a wild-type strain and an insecticide-resistant strain were presented. Results were negative for 4-Nitro-o-Phenylenediamine in both strains.

DNA Synthesis-Inhibition Test

Heil and Reifferscheid (1992) evaluated 2-Nitro-p-Phenylenediamine and other compounds in an immunological DNA synthesis-inhibition test. DNA-synthesizing cells were fed with the thymidine analog 5-bromodeoxyuridine (BrdU), which is incorporated specifically into DNA. Incorporated BrdU was determined by standard immunological techniques. DI_{50} (i.e., the concentration of an agent in mol/l that inhibits DNA synthesis by 50%) values were presented. 2-Nitro-p-Phenylenediamine did not inhibit DNA synthesis.

MUTAGENICITY ENHANCEMENT/INHIBITION

Ames Test

4-Nitro-o-Phenylenediamine and 2-Nitro-p-Phenylenediamine

In a study by Lee et al. (1988), various concentrations of butylated hydroxyanisole had significant effects on the mutagenic activity induced by 4-Nitro-o-Phenylenediamine and 2-Nitro-p-Phenylenediamine (in *Salmonella typhimurium* strain TA98) at the same concentration of 0.46 µM. Butylated Hydroxyanisole (BHA, 0 to 0.46 µM) decreased the mutagenicity of 4-Nitro-o-Phenylenediamine in a dose-response manner in the absence of metabolic activation. In the presence of metabolic activation, the mutagenicity of 2-Nitro-p-Phenylenediamine was significantly inhibited by BHA, but the mutagenicity of 4-Nitro-o-Phenylenediamine was significantly increased. The inhibitory effects of BHA on the mutagenicity of 2-Nitro-p-Phenylenediamine were more potent than those of 4-Nitro-o-Phenylenediamine.

In the Ames test, neither of the four compounds had an antimutagenic effect on

2-Nitro-p-Phenylenediamine (dose = 30 µg/plate) or 4-Nitro-o-Phenylenediamine (dose = 10 µg/plate): tannic acid (up to 0.2 µmol), propyl gallate (up to 0.2 µmol), ellagic acid (up to 0.2 µmol), and gallic acid (up to 0.2 µmol) (Chen and Chung, 2000).

Using *Salmonella typhimurium* strain TA98, Gentile et al. (1985) found that 4-Nitro-o-Phenylenediamine was activated in vitro by pea or tobacco S-9 into a more potent mutagen. A direct relationship between plant peroxidase and 4-Nitro-o-Phenylenediamine activation was established. At the highest concentration tested (39.1×10^{-8}), the mean number of revertants per plate was 765 ± 23.4 without activation, 3,261 ± 72.4 with pea S-9, 2,932 ± 94.7 with tobacco S-9, and 639 ± 17.2 with mammalian hepatic S-9. The plate-incorporation assay by Maron and Ames (1983) was used.

Sister Chromatid Exchange Assay

4-Nitro-o-Phenylenediamine and 2-Nitro-p-Phenylenediamine

The effects of BHA on sister chromatid exchange frequencies (Chinese hamster ovary cells) induced by 4-Nitro-o-Phenylenediamine and 2-Nitro-p-Phenylenediamine were evaluated in a study by Lee et al. (1988). In the absence of metabolic activation, the sister chromatid exchange frequencies induced by 2-Nitro-p-Phenylenediamine were decreased by BHA, but the sister chromatid frequencies of 4-Nitro-o-Phenylenediamine were not decreased. In the presence of metabolic activation, BHA did not decrease the sister chromatid exchange frequencies that were induced by 2-Nitro-p-Phenylenediamine. After the culture was treated with 4-Nitro-o-Phenylenediamine and BHA, (both at 0.4 mM concentration), no metaphase cells were harvested.

CARCINOGENICITY

2-Nitro-p-Phenylenediamine

2-Nitro-p-Phenylenediamine (1,4-Diamino-2-Nitrobenzene) was considered by a previous International Agency for Research on Cancer (IARC) Working Group (IARC, 1978). Since that time, new data have become available, and these data have been incorporated into the monograph and taken into consideration in this IARC evaluation (IARC, 1993): Evaluation: There is inadequate evidence in humans for the carcinogenicity of 1,4-diamino-2-nitrobenzene. There is limited evidence in experimental animals for the carcinogenicity of 1,4-diamino-2-nitrobenzene. Overall evaluation: 1,4-Diamino-2-nitrobenzene is not classifiable as to its carcinogenicity to humans (Group 3).

CLINICAL ASSESSMENT OF SAFETY

SKIN SENSITIZATION

2-Nitro-p-Phenylenediamine

A study of cosmetic intolerance was undertaken by Broeckx et al. (1987) using 5,202 patients, 3330 women (64%) and 1872 men (36%), being tested for contact dermatitis, using computer analysis of extensive medical histories and epicutaneous tests. Of the total number of patients suffering from cosmetic intolerance, 93 (1.8%) had an allergic reaction to 2-Nitro-p-Phenylenediamine. Of the patients exhibiting a pure allergy, without irritation, only to cosmetics, five (3.2%) had an allergic reaction to 2-Nitro-p-Phenylenediamine.

Guerra et al. (1992) performed a multicenter study in 9 Italian centers by members of the Gruppo Italiano Ricercaq Dermatiti da contatto e Ambientali (GIRDCA)

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. Occupational habits and use of preventive measures were specifically investigated both in these 302 hairdressers and in a further group of 240 hairdressers who answered a questionnaire. The results showed the presence of an occupationally relevant sensitization in 60.9% of the 302 hairdressers. This proportion included 52 hairdressers who had negative patch tests to the hairdressers' series but showed positive reactions to other allergens, such as nickel, rubber, additives, preservatives and fragrances, which were judged relevant to their occupation. Among hair dyes, 2-Nitro-p-Phenylenediamine (1% in pertrolatum) caused 24 sensitization reactions (7.9%).

To obtain data on the frequency of sensitization among European hairdressers, the patch test results from 9 centers were evaluated by Frosch et al. (1993). A total of 809 hairdressers and 104 clients suspected of contact sensitization were patch tested. Among hairdressers, the mean frequency of sensitization to 2-Nitro-p-Phenylenediamine (1% in petrolatum) was 4%. The incidence of positive patch test reactions to 2-Nitro-p-Phenylenediamine in hairdressers' clients was 7.7%.

Goossens et al. (1999) reported a retrospective European 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 in five European dermatology centers. A total of 8 allergic reactions to 2-Nitro-p-Phenylenediamine was reported.

Fautz et al. (2002) investigated the cross-reaction pattern of new generation hair dyes among 40 hairdressers (from hairdressers' clinic in the Netherlands) with a known

allergy to 4-phenylenediamine, and/or 2,5-diaminotoluene sulfate and/or 2-Nitro-p-Phenylenediamine. In new generation hair dyes, FD & C and D & C dyes are used in hair dye formulations. In the 40 hairdressers, no positive reactions were observed to the single FD & C and D & C dyes. In two hairdressers, doubtful reactions to one or more of the hair dye formulations were observed. The data from this study suggest that for hairdressers sensitized to 4-phenylenediamine and/or 2,5-diaminotoluene sulfate and/or 2-Nitro-p-Phenylenediamine, this new generation of hair dyes is a safe alternative for use in their salons.

CASE REPORT

In a case report by Van Joost et al. (1987), a toxic allergic skin reaction of the face, neck, and arms (due to accidental contact with methylenedianiline [also known as 4-4'-diaminodiphenylmethane]) was observed in a 32-year-old cleaner at a chemical plant. Patch test results for 2-Nitro-p-Phenylenediamine were positive at 48 hours (+++ reaction) and 72 hours (+++ reaction).

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