Amended Safety Assessment of 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate as Used in Cosmetics

Status:	Draft Amended Report for Panel Review
Release Date:	September 6, 2024
Panel Meeting Date:	September 30 - October 1, 2024

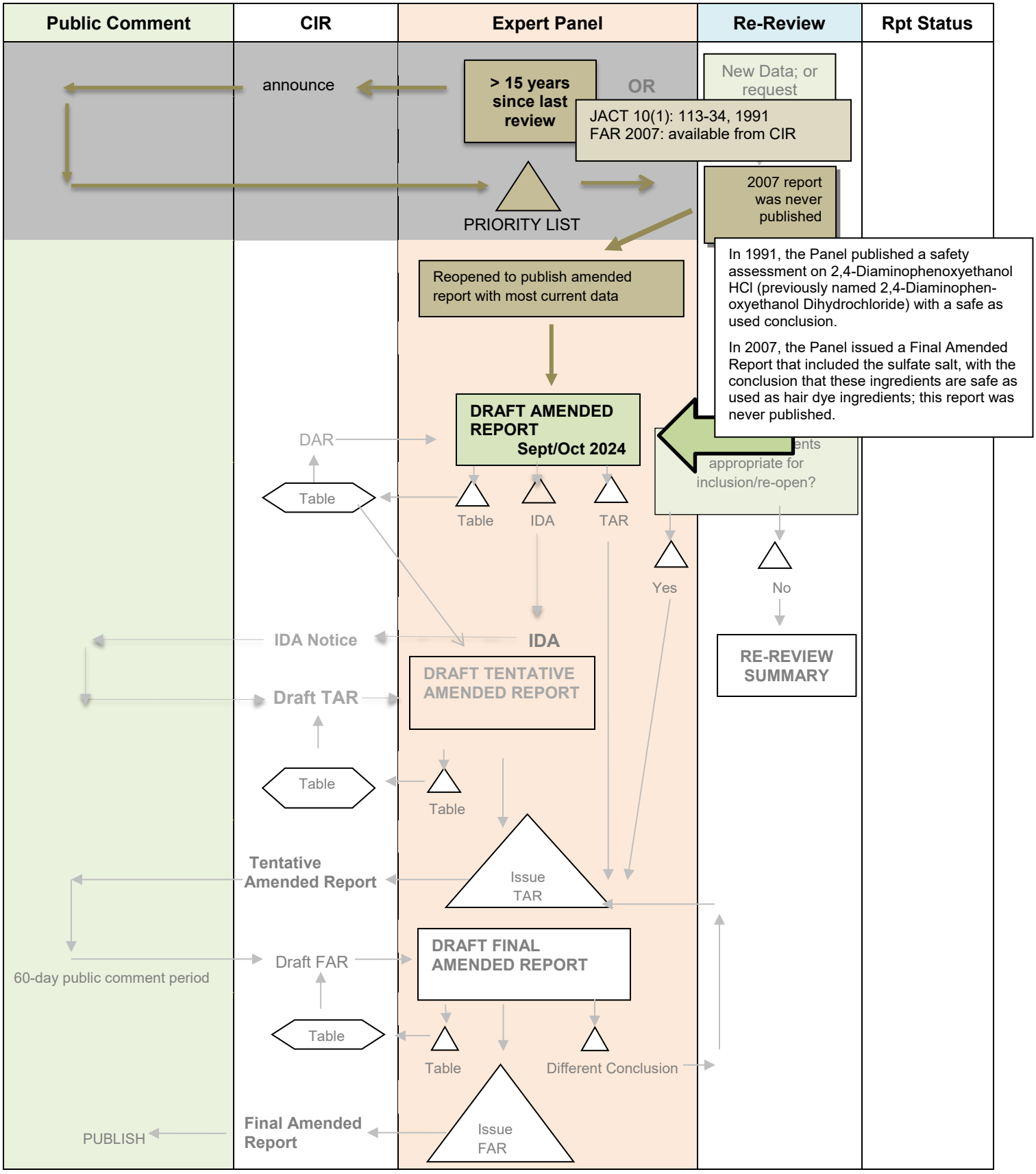
The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D.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

555 13th Street, NW, Suite 300W, Washington, DC 20004
cirinfo@cir-safety.org

RE-REVIEW FLOW CHART

INGREDIENT/FAMILY 2,4-Diaminophenoxyethanol HCl and Sulfate

MEETING September/October 2024



Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, M.S., Senior Scientific Analyst/Writer, CIR
Date: September 6, 2024
Subject: Amended Safety Assessment of 2,4-Dimainophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate as Used in Cosmetics

Enclosed is the Draft Amended Report on the Amended Safety of 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate as Used in Cosmetics. (It is identified as *report_Diaminophenoxyethanol_092024* in the pdf document). In 1991, the Panel published a safety assessment on 2,4-Diaminophenoxyethanol HCl (previously named 2,4-Diaminophenoxyethanol Dihydrochloride) with the conclusion that "2,4-Diaminophenoxyethanol Dihydrochloride is safe as a cosmetic ingredient in the present practices of use and concentration" (*1991-originalreport_Diaminophenoxyethanol_092024*). In 2007, the Panel issued a Final Amended Report that included the sulfate salt, with the conclusion that these ingredients are safe as hair dye ingredients in the practices of use and concentration described in the report (*2007-amendedreport_Diaminophenoxyethanol_092024*); this report was never published. Because more than 15 years have passed since the Panel last reviewed this report and because the 2007 report was never published, it is now time to review these ingredients again to update the information and then publish this amended safety assessment on 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate. New data have been incorporated into the existing 2007 report, but the data from the 1991 report that were originally included as part of the 2007 document are now summarized in *italics*.

According to 2023 VCRP survey data, 2,4-Diaminophenoxyethanol HCl is reported to be used in 93 formulations. The majority of these uses are in hair coloring preparations; however, uses have been reported for eye makeup preparations. Ten (10) uses were reported 2,4-Diaminophenoxyethanol Sulfate; 1 of these uses is reported in an eye makeup preparation. The frequencies of use for 2,4-Diaminopheoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate have only slightly changed since these ingredients were last reviewed by the Panel. In 2006, 2,4-Diaminophenoxyethanol HCl was reported in the VCRP to be used in 115 formulations and 2,4-Diaminophenoxyethanol Sulfate was reported to be used in 5 formulations. At that time, all uses for both ingredients were reported to be in hair coloring formulations.

The results of the concentration of use survey conducted by the Council in 2022 (*data_ Diaminophenoxyethanol_092024*) indicate 2,4-Diaminophenoxyethanol HCl has a maximum concentration of use range of 0.56 - 2.4% in hair dyes. 2,4-Diaminophenoxyethanol Sulfate has a maximum concentration of use range of 0.25 - 0.35% in hair dyes. In the 2007 amended report, the maximum concentration of use range for 2,4-Diaminophenoxyethanol HCl was 0.05 - 2% in hair dyes; the sulfate salt was reported to be used at a maximum concentration of 0.4 - 2% in hair dyes. (The 2% is the final on-head concentration after mixing with hydrogen peroxide for both ingredients).

Additional supporting documents for this report package include a flow chart (*flow_ Diaminophenoxyethanol_092024*), report history (*history_ Diaminophenoxyethanol_092024*), a search strategy (*search_ Diaminophenoxyethanol_092024*), a data profile (*dataprofile_Diaminophenoxyethanol_092024*), and the minutes from all the meetings at which 2,4-Diaminophenoxylethanol HCl and 2,4-Diaminophenoxyethanol Sulfate were discussed during the original review (*originalminutes_Diaminophenoxyethanol_092024*).

If no further data are needed, the Panel should formulate an updated Discussion and issue a Tentative Amended Report. However, if additional data are required, the Panel should be prepared to identify those needs and issue an Insufficient Data Announcement.

2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate History

1991– The CIR Final Report on the Safety Assessment of 2,4-Diaminophenoxyethanol HCl (then named 2,4-Diaminophenoxyethanol Dihydrochloride) was published in the *Journal of the American College of Toxicology*. The Panel concluded that 2,4-Diaminophenoxyethanol HCl is safe as a cosmetic ingredient in the present practices of use and concentration.

June 2007 – After a re-review of the available literature was performed, the Panel re-opened the safety assessment on 2,4-Diaminophenoxyethanol HCl to add 2,4-Diaminophenoxyethanol Sulfate.

September 2007 – The Panel reviewed the Draft Amended Report and concluded that 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are safe as hair dye ingredients in the present practices of use and concentration as described in the safety assessment.

December 2007 – The Panel issued a Final Amended Report with the conclusion that 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are safe as hair dyes in the practices of use and concentration described in the safety assessment.

July 2024 – Review of the available published literature since 2007 was conducted in accordance to CIR Procedures regarding re-review of ingredients after ~15 years. Because the document was not published in the *International Journal of Toxicology* after the Panel finalized the Amended Report in 2007, the document is being presented to the Panel as a Draft Amended Report and is incorporating the data from that report along with newly available data.

2,4-Diamionphenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate Data Profile* – September 2024 – Christina Burnett																															
				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci			Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	Other	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
2,4-Diaminophenoxyethanol HCl; CAS No. 66422-95-5	X O		X O	X	X O	X		X O			X O		O	X O	X O	X O		O			X O	X O		X O				X O		X
2,4-Diaminophenoxyethanol Sulfate; CAS No. 70643-20-8	X O		X	X																											

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

2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SC
2,4-Diaminophenoxyethanol HCl	66422-95-5	√	√	√	√	√	√	√	√	√	√	√	√
2,4-Diaminophenoxyethanol Sulfate	70643-20-8	√	√	√	√	√	√	√	√	√	√	√	√

Search Strategy

Pub Med – Search from 2005 to present

((2,4-Diaminophenoxyethanol HCl) OR 66422-95-5[EC/RN Number]) – 2 hits, 1 useful

((2,4-Diaminophenoxyethanol Sulfate) OR 70643-20-8[EC/RN Number]) -2 hits, 1 useful (same as above)

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/

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/

APRIL 16-17, 1990 PANEL MEETING – FIRST OPEN REVIEW

Dr. Bergfeld opened the discussion, indicating that the compound was referred to as 2,4-DAPE. She noted that the compound was used in oxidative hair dye, and appeared in 82 product formulations. She noted that the majority of the uses were at concentrations ≤ 0.1%. She remarked that the compound appeared to be non-mutagenic and non-carcinogenic, and that animal studies suggested some slight irritation. She brought to the Panel's attention the lack of human data, and the fact that this ingredient was a hair dye subject to the Food, Drug, and Cosmetic Act of 1938. She indicated that, with the appropriate clarification and statements regarding the lack of human data in the report, the conclusion should be safe as used for 2,4-DAPE. She commented that, as in the previously discussed ingredient, there was a problem with the ocular data in the Discussion. She asked that a summary of irritation and sensitization data in animals be added.

Dr. Carlton noted that the discussion would have to be rewritten as the previous ingredient had been.

Dr. Bergfeld concurred, and moved to accept the document with the addition to the summary and the changes in the discussion. She then opened the discussion for comments from the Panel.

Dr. Schroeter supported the addition to the summary, as well as the deletions in the discussion, and seconded the motion.

Mr. Eiermann indicated that there was not enough information in the Cosmetic Use section on the actual chemical reactions taking place in cosmetic formulations, the only information provided was strictly concentrations of use.

Dr. Berndt asked if it would be possible for the writer to look up some of the chemistry and add it to the report. He asked for other comments on the report.

Dr. Hoffmann indicated that the chemical structure of 2,4-DAPE should include brackets around the HCl portion of the structure, or that it be represented by writing dihydrochloride instead of · HCl.

Dr. McEwen concurred.

Dr. Berndt asked for other comments from the Panel. He called for a vote to approve the document as safe as used with the changes noted. The motion was carried unanimously.

JUNE 4-5, 2007 PANEL MEETING – RE-REVIEW

Belsito's Team

Dr. Belsito noted that there was no new data for this ingredient.

Industry representatives noted that there is new data in form of the dossier submitted to Europe.

Dr. Belsito asked the team if they felt the sulfate salt should be added to the report.

The team responded that the sulfate salt should be added.

Dr. Belsito stated the report should be re-opened to add the sulfate salt of 2,4-Diaminophenoxyethanol. New data from the European dossier should be added during this time.

Marks' Team

Dr. Marks introduced the ingredient to the team by stating that in 1991, the CIR Expert Panel concluded that 2,4-Diaminophenoxyethanol Dihydrochloride was safe as used. There currently is no new data on the ingredient. He asked the team if the report should be re-opened to add the sulfate salt.

Drs. Shank and Slaga replied that the report should be re-opened to add the sulfate salt.

Dr. Marks stated that the ingredient will go through the review re-open process.

Dr. Bergfeld stated that the report will become a Tentative Final Safety Assessment.

Dr. Andersen said that the report would become a Draft Report.

Dr. Marks expected that the conclusion will be "safe as used" when the Panel sees the Tentative Draft.

Dr. Andersen reminded the team that data could always come out of left field.

Dr. Bergfeld was concerned about the consistency of the re-review process. Re-open to add ingredient is not consistent with re-open to add new safety data.

Dr. Andersen replied that it is possible that new data could still be presented.

Dr. Marks asked Ms. Burnett if new data was searched for both 2,4-Diaminophenoxyethanol and the sulfate salt.

Ms. Burnett replied yes, both were researched.

Dr. Marks stated the team will move to re-open the ingredient to add the sulfate salt.

Full Panel Meeting

Dr. Marks stated that a CIR Final Report with the following conclusion was published in 1991: On the basis of the data presented in this report, the CIR Expert Panel concludes that 2,4-Diaminophenoxyethanol Dihydrochloride is safe as a cosmetic ingredient in the present practices of use and concentration.

Dr. Marks added that the results of a literature search did not identify any new studies in the published literature since the Final Report was issued, and that there are no new concerns relating to the safety of this ingredient in cosmetics. However, he noted that his Team recommended that the Final Report be reopened to add the sulfate salt, and expects that there will be no safety issues relating to this ingredient.

Dr. Belsito said that the Panel was informed that additional data on the sulfate salt are expected from the cosmetics industry.

The Panel voted unanimously in favor of reopening the Final Report on 2,4-Diaminophenoxyethanol Dihydrochloride to add 2,4-Diaminophenoxyethanol Sulfate to the safety assessment.

SEPTEMBER 2007 PANEL MEETING – DRAFT A\AMENDED REPORT

Full Panel Meeting

Dr. Marks stated that a CIR Final Report with a safe as used conclusion on 2,4-Diaminophenoxyethanol Dihydrochloride (now 2,4-Diaminophenoxyethanol HCl) was published in 1991, and, at the April 16-17, 2007 Panel meeting, this conclusion was reconfirmed and the Panel decided not to reopen the final report. He added that his Team is now proposing that the Final Report be reopened to add 2,4-Diaminophenoxyethanol Sulfate, after considering that the safe as used conclusion is also applicable to this ingredient. Thus, it was agreed that a Tentative Amended Final Report including the sulfate salt should be issued.

Dr. Bergfeld asked if there are any changes in the report text that need to be made.

Dr. Marks said that the following statement should be deleted from the report summary: A few assays that did observe genotoxicity were discounted. He added that the following statement should be deleted from the report discussion: Based on the in vitro dermal penetration study of this ingredient as used in simulated oxidative hair dyes, 90% is washed off the skin surface at the time when the hair would be rinsed, so it is unlikely that, in practice, hair dyes containing this ingredient would cause dermal sensitization.

Dr. Belsito said that the following statement relating to hair dye epidemiology should also be deleted from the discussion: Hair dye epidemiology data are expected to broadly apply to both ingredients. The CIR Expert Panel concluded that the available epidemiology studies are sufficient to conclude there is a causal relationship between hair dye use and cancer and other endpoints. The Panel recognizes that hair dye epidemiology studies do not address the safety of individual hair dyes, but is concerned that studies have demonstrated an association between use of oxidative permanent hair dyes and some cancer endpoints. The Panel, therefore, strongly supports the need to replicate these studies, along with further studies to examine the possibility of susceptible subpopulations.

Dr. Belsito said that it has not been the practice of the Panel to include statements relating to hair dye epidemiology in the discussion section of CIR reports on hair dyes. He noted that the hair dye epidemiology section of the report precedes the summary, and that there is no need to reiterate or summarize this information.

Dr. Marks recommended that the report conclusion be revised so that it conforms with the current boilerplate conclusion for CIR reports.

The Panel voted unanimously in favor of issuing a Tentative Amended Final Report with the following conclusion: On the basis of data presented in this report, the CIR Expert Panel concludes that 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are safe as hair dye ingredients in the present practices of use and concentration as described in this safety assessment.

DECEMBER 10-11, 2007 PANEL MEETING – DRAFT FINAL AMENDED REPORT

Dr. Marks stated that a CIR final report with a conclusion stating that 2,4-Diaminophenoxyethanol Dihydrochloride (now known as 2,4-Diaminophenoxyethanol HCl) is safe as a cosmetic ingredient in the present practices of use and concentration was published in 1991. He added that, at the September 2007 Panel meeting, the final report was reopened to include 2,4-Diaminophenoxyethanol Sulfate and a tentative amended final report with the following conclusion was issued: On the basis of

data presented in this report, the CIR Expert Panel concludes that 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are safe as hair dye ingredients in the present practices of use and concentration as described in this safety assessment.

Dr. Marks noted that his Team is in favor of issuing an Amended Final Report with the preceding conclusion.

Dr. Belsito said that his Team is requesting deletion of the last paragraph of the report summary and discussion and the replacement of deleted text with the new paragraph from the hair dye epidemiology boilerplate, which reads as follows: The CIR Expert Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between current hair dye use and cancer and other endpoints based on lack of strength of the association and inconsistency of findings.

The Panel agreed with the Belsito Team's changes in the report discussion and summary and voted unanimously in favor of issuing an amended final report with the following conclusion: On the basis of the data presented in this report, the CIR Expert Panel concludes that 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are safe as hair dye ingredients in the present practices of use and concentration as described in this safety assessment.

Amended Safety Assessment of 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate as Used in Cosmetics

Status: Draft Amended Report for Panel Review
Release Date: September 6, 2024
Panel Meeting Date: September 30 - October 1, 2024

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

555 13th Street, NW, Suite 300W, Washington, DC 20004
cirinfo@cir-safety.org

ABBREVIATIONS

CIR	Cosmetic Ingredient Review
CPSC	Consumer Product Safety Commission
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
EC_3	estimated concentrations of an SI of 3
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FD&C Act	Food, Drug, and Cosmetic Act
DMSO	dimethyl sulfoxide
LLNA	local lymph node assay
MOE	margin of exposure
MOS	margin of safety
NOAEL	no-observed-adverse-effect level
NR	not reported
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PCPC	Personal Care Products Council
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dose
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SI	stimulation index
SPF	specific pathogen-free
TG	test guideline
US	United States
VCRP	Voluntary Cosmetic Registration Program

INTRODUCTION

This assessment reviews the safety of 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), these ingredients are reported to function as hair colorants in cosmetic products.[1]

The Expert Panel for Cosmetic Ingredient Safety (Panel) first reviewed the safety of 2,4-Diaminophenoxyethanol HCl (previously named 2,4-Diaminophenoxyethanol Dihydrochloride) individually in a report published in 1991, with the conclusion "2,4- Diaminophenoxyethanol Dihydrochloride is safe as a cosmetic ingredient in the present practices of use and concentration."[2] In a 2007 re-review, the 2,4-Diaminophenoxyethanol HCl report was reopened to add 2,4-Diaminophenoxyethanol. That amended report was finalized in the same year with the conclusion that these ingredients are safe as hair dyes in the practices of use and concentration as described in the safety assessment, but the amended report was never published.[3] Because it had been at least 15 years since the Panel reviewed these ingredients last and because the 2007 report was never published, in accordance with the Cosmetic Ingredient Review (CIR) Procedures, the Panel considered the updated information available in 2024. This current amended report on 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate is an updated version of the 2007 assessment, and includes all studies considered in the 2007 amended report as well as new studies available since then. Excerpts from the summaries of the 1991 report are disseminated throughout the text of this document, as appropriate, and are identified by italicized text.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; this search was last performed August 2024. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cirsafety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Some chemical and toxicological data on 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate included in this safety assessment were obtained from opinions produced by the Scientific Committee on Consumer Products (SCCP) and Scientific Committee on Consumer Safety (SCCS) of the European Commission.[4,5] Additionally, data were obtained from robust summaries of data submitted to the European Chemicals Agency (ECHA) by companies as part of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) chemical registration process.[6,7] These data summaries are available on the databases for ECHA and the European Commission, respectively, and when deemed appropriate, information from the summaries has been included in this report.

CHEMISTRY

Definition and Structure

2,4-Diaminophenoxyethanol HCl (CAS No. 66422-95-5) and 2,4-Diaminophenoxyethanol Sulfate (CAS No. 70643-20-8) are the aromatic amine salts that conform to the structures in Figure 1.[8]

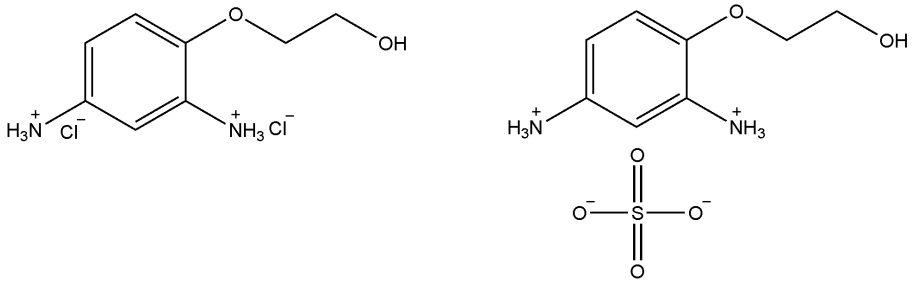


Figure 1. 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate

Both of these ingredients function as *m*-diamine couplers in oxidative (permanent) hair colorant products. In oxidative hair colorant products, couplers such as 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate act as nucleophiles which are reacted with ingredients called precursors (which are activated by an oxidizing agent, typically hydrogen peroxide). The products of the reactions between couplers and activated precursors comprise the actual hair dye molecules. These hair dye molecules work, in part, by penetrating the hair shaft.

Chemical Properties

Chemical properties for 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are summarized in Table 1. 2,4-Diaminophenoxyethanol HCl is a light grey to light pink or lavender powder with a formula weight of 241 g/mol and a log P_{ow} value of 0.99.[2,4,5] 2,4-Dimainophenoxyethanol Sulfate is a white powder with a formula weight of 266.27 (302.3 as a dihydrate) and a log P_{ow} value of -0.612 (as dihydrate).[4,5]

Method of Manufacture

According to data submitted to the SCCS, 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are synthesized via the same route.[5] No further information was provided to the SCCS. No other method of manufacture data were found in the published literature, and unpublished data were not submitted.

Impurities

1,3-Diaminobenzene; 2,4-diamino-1-methoxybenzene; and 2,4-diamino-1-ethoxybenzene are not detected as impurities in 2,4-Diaminophenoxyethanol HCl.[2]

The purity of 3 different batches of 2,4-Diaminophenoxyethanol HCl ranged from 98.7 to 99.4%.[4] In analysis of the batch that was 98.7% pure, *m*-phenylenediamine was < 100 ppm; 2,4-diaminoanisole was not detected (detection limit = 100 ppm); and isopropanol was < 100 ppm. Heavy metals content was as follows: arsenic, antimony, and mercury < 5 ppm; cadmium < 10 ppm; and lead < 20 ppm.

The purity of 5 different batches of 2,4-Diaminophenoxyethanol Sulfate (dihydrate) ranged from 98.2 to 100% (254 nm; area %).[5] In the analysis of the 5 batches, *m*-phenylenediamine content was 8 - 14 ppm and 2,4-diaminoanisole content was 13 - 15 ppm (in 3 of 5 batches, the remaining 2 were below the limit of detection of 10 ppm). Iron and other heavy metals were < 10 ppm in at least 4 batches (one did not report heavy metals and the other reported iron to be 190 ppm). The SCCS commented that based on the information that 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are synthesized via the same route, similar impurity profiles should be expected for both materials.

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics, and does not cover their use in airbrush delivery systems. Data included herein were obtained from the FDA's Voluntary Cosmetic Registration Program (VCRP) database in 2023 (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data were provided by cosmetic product categories, based at that time on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP survey data, 2,4-Diaminophenoxyethanol HCl is reported to be used in 93 formulations (89 of which are in hair coloring preparations and 4 are in eye makeup preparations) and 2,4-Diaminophenoxyethanol Sulfate is reported to be used in 10 formulations (9 of which are in hair coloring preparations and 1 is in an eye makeup preparation) (Table 2).[9] The frequencies of use for these ingredients have only slightly changed since these ingredients were last reviewed by the Panel. In 2006, 2,4-Diaminophenoxyethanol HCl was reported to be used in 115 formulations and 2,4-Diaminophenoxyethanol Sulfate was reported to be used in 5 formulations.[3] All uses for these ingredients were in hair dyes and coloring products. The results of the concentration of use survey conducted by the Council in 2022 indicate 2,4-Diaminophenoxyethanol HCl has a maximum concentration of use range of 0.56 - 2.4% in hair dyes.[10] 2,4-Diaminophenoxyethanol Sulfate has a maximum concentration of use range of 0.25 - 0.35% in hair dyes. In the 2007 amended report, the maximum concentrations of use range for 2,4-Diaminophenoxyethanol HCl was 0.05 - 2% in hair dyes; the sulfate salt was reported to be used at a maximum concentration of 0.4 - 2% in hair dyes (2% is the final on-head concentration after mixing with hydrogen peroxide for both ingredients).[3]

These ingredients are considered coal tar hair dyes for which regulations require caution statements and instructions regarding patch tests in order to be exempt from certain adulteration and color additive provisions of the 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.

However, 4 of the reported uses of 2,4-Diaminophenoxyethanol HCl and 1 of the reported uses of 2,4-Diaminopheoxyethanol Sulfate have been reported for eye makeup preparations. 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are exempt from certain adulteration and color additive provisions of the FD&C Act only when used as coal tar hair dye ingredients. With regard to the reported use in eye makeup preparations, the FD&C Act mandates that color additives must be approved by FDA for their intended use before they are used. 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are not approved color additives in non-hair dye

cosmetic products, and thereby, use in eye makeup products is not permitted. Furthermore, as stated above, the FD&C Act also specifies that coal tar hair dyes must not be used for dyeing the eyelashes or eyebrows.

Product labels shall also bear patch test instructions for determining whether the product causes skin irritation. However, whether or not patch testing prior to use is appropriate is not universally agreed upon. The Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 h after application of the test material and prior to the use of a hair dye formulation. Conversely, a report in Europe suggests that self-testing has severe limitations, and may even cause morbidity in consumers.[11,12] Hair dye products marketed and sold in the US, though, must follow the labeling requirements established by the FD&C Act.

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

Under European regulations for cosmetic ingredients, 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are listed in Annex III with the restrictions that these ingredients may be used as hair dyes substances in oxidative hair dye products.[13] Additionally, these ingredients may be used in products intended for coloring eyelashes. The SCCS has determined that the use of 2,4-Diaminophenoxyethanol HCl or 2,4-Diamionphenoxyethanol Sulfate in oxidative hair dye formulations at a maximum final concentration of 2.0% (after mixing with hydrogen peroxide) does not pose a risk to the health of the consumer, apart from its sensitizing potential.[5]

TOXICOKINETIC STUDIES

Dermal Penetration

In Vitro

The percutaneous absorption of 2,4-Diaminophenoxyethanol HCl was determine using human dermatomed skin (370-400 µm thick) mounted on diffusion cells.[3,4] This study was performed in accordance with the Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 428. The radioactive tracer 2-(2,4-Diamino[ring-U-^{14}C]-phenoxyethanol hydrochloride (98.6% radiochemical purity) was added to 98.7% pure 2,4-Diaminophenoxyethanol HCl. The radiolabeled test material (0.41%) and the unlabeled test material (3.59%) was then studied in oxidative and non-oxidative formulations. In the oxidative hair dye simulation, immediately prior to application, equal parts of the oxidative formulation and the developer (6% hydrogen peroxide) were combined, yielding a final concentration of 2.0% 2,4-Diaminophenoxyethanol HCl. In the non-oxidative hair dye simulation, immediately prior to application, equal parts of non-oxidative formulation containing 4% 2,4-Diaminophenoxyethanol HCl and degassed water were combined. Absorption was assessed by collecting the phosphate buffered saline receptor fluid hourly from 0 to 24 h. At 30 min after sample application, the skin was washed with water, 2% sodium dodecyl sulfate (w/v in water), and water again. At 24 h, the underside of the skin was rinsed with receptor fluid. The skin was removed from its mounting, dried, and tape-stripped. Samples were analyzed using liquid scintillation counting. A total of 12 skin samples were tested for both simulations, but data from 4 of the oxidative hair dye simulations were rejected due to low mass balance (no further details provided). The dermal delivery (sum of absorbed dose + the mass of test material in the dermis/epidermis) was reported to be 1.74 µg equivalents/cm^2 (0.41% of the applied dose) for the oxidative formulation and 6.55 µg equivalents/cm^2 (1.68% of the applied dose) for the non-oxidative formulation. However, the SCCP stated the maximum dermal absorption of 4.33 µg equivalents/cm^2, observed under oxidative conditions in a typical hair dye formulation, would be used for the calculation of the margin of exposure (MOE).

Animal

Dermal absorption of [^{14}C]2,4-Diaminophenoxyethanol HCl was determined in female rats using pure compound and in a formulation at 0.40%.[2] *Both the pure compound and the formulation were dissolved in a vehicle containing nonionic, and amphoteric surfactants, alcohols, glycols, oleic acid, copra diethanolamine, antioxidants, completing agents, water, and 10% aqueous ammonia. Prior to application the solution was mixed with an equal volume of 20% hydrogen peroxide solution and 20 mg/cm^2 of the compound was applied to a 25 cm^2 area of the dorsal region of the rats for 40 min. Excess material was removed following the exposure period. Urine and feces were collected for 4 d after treatment. Animals were then killed and necropsy was performed to determine how much of the test material was absorbed. For the pure compound, 5.05 $\pm$ 0.79 nM/cm^2 penetrated the skin. In formulation, 2.83 $\pm$ 0.49 nM/cm^2 penetrated the skin.*

This study was then continued in additional animals by applying the hair dye solutions every 30 - 40 d (total number of iterations not provided) to simulate human hair dying frequency.[2] *The livers and thyroids of the treated rats were examined for accumulation of the test material. At the highest doses, with the animals killed 4 d after treatment, no radioactivity was present in the thyroid, and only trace amounts were present in the liver.*

In another study, female rats received dermal applications of [^{14}C]2,4-Diaminophenoxethanol HCl dissolved in a commercial vehicle at concentrations of 0.40, 0.80, or 1.20% (23.65, 47.30, or 70.95 nM, respectively).[2] *The solutions was*

mixed with an equal volume of 20% hydrogen peroxide before application. Groups of 6 rats received 20 mg/cm^2 on an area of 25 cm^2 for 40 min. Urine and feces were collected for 4 d after treatment before the animals were killed and necropsied to determine how much of the test material was absorbed. At 0.40%, 5.03 $\pm$ 0.79 nM/cm^2 penetrated the skin, and at 1.20%, 9.42 $\pm$ 0.84 nM/cm^2 penetrated the skin.

Absorption, Distribution, Metabolism, and Excretion (ADME)

Animal

Oral

As part of a 13-wk subchronic toxicity study, groups of 6 male and 6 female Sprague-Dawley rats received 4, 20, or 100 mg/kg/d 2,4-Diaminophenoxyethanol HCl in 5 ml purified water via gavage for toxicokinetic evaluation performed on day 1 and during week 13 (results of the toxicological study are described below in the Subchronic Toxicity section).[3,4] Plasma levels of 2,4-diaminophenoxyethanol were not detectable in the low-dose group. In the 20 mg/kg/d group, plasma levels of 2,4-diaminophenoxyethanol were detectable at 30 min after dosing (no shorter determination was made). In the 100 mg/kg/d dose group, plasma levels of 2,4-diaminophenoxyethanol were maximal at 30 min. Thereafter, the plasma levels decreased steadily until the last quantifiable time-point (2 h on day 1, 8 h in week 13). There were no differences observed between the sexes. Systemic exposure increased with dose-level in a proportional manner, and mean area under the curve (AUC_{0-t}) values of 5.22 and 7.40 µg·h/ml were achieved in week 13 for male and females given 100 mg/kg/d, respectively.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

In an acute gavage study of male and female albino Swiss mice and male and female Wistar rats, the LD_{50} for 2,4-Diaminophenoxyethanol HCl (1 ml/100 g; chlorhydrate form) was determined to be 1745 mg/kg for mice and 1113 mg/kg for rats (dose concentrations calculated in geometric progression, no further details provided).[2] In a gavage study of male albino Swiss mice only, the LD_{50} for 2,4-Diaminophenoxyethanol HCl was determined to be 1160 mg/kg. The mice received the test material at concentrations ranging from 630 - 2500 mg/kg in a dose volume of 10 ml/kg of the compound either dissolved in water or suspended in 0.5% hydroxypropyl methylcellulose..

Fasted Sprague-Dawley rats (5 male and 5 females) received a single dose of 1000 mg/kg 2,4-Diaminophenoxyethanol HCl (99.6% pure) in water by gavage (10 ml/kg).[3,4] This acute oral toxicity study was performed in accordance with OECD TG 401. During the 14-d observation period, 1 male and 3 female rats were found dead on day 2. Death was preceded by hypoactivity, piloerection, lateral decubitus, and tonic-clonic convulsions, all of which were also observed in the surviving animals. All surviving animals recovered fully by day 5. No abnormalities were observed at necropsy in either the animals that died during the study or those surviving until the end of the observation period. It was determined that the LD_{50} was close to 1000 mg/kg bw in this study.

Subchronic Toxicity Studies

Oral

In a 12-wk study, groups of 10 male and 10 female BDF_1 mice and 10 male and 10 female F344 rats received 0, 0.01. 0.03, 0.05, 0.1, or 0.2% 2,4-Diaminophenoxyethanol HCl in tap water.[2] No clinical signs of toxicity were observed during treatment. Reduced body weight gains were observed in male mice of the 0.1 and 0.2% dose groups, but these effects were not observed in the female mice. Feed consumption was reduced in the 0.1 and 0.2% dose groups for the male and female mice. In rats, a dose-dependent decrease in mean body weight gain was observed in all treatment groups and feed consumption was reduced in the 0.2% dose group. In the 0.2% dose group, abnormalities of the kidneys were found in 2 mice, lesions of pneumonia were found in 7 mice, and pigment deposits were observed in the epithelial cells of thyroid follicles in 1 mouse. All male and female rats in the 0.2% dose group had pigment deposits in the epithelial cells of the thyroid follicles. There was no evidence of silver or iron in the deposits in either mice or rats.

2,4-Diaminophenoxyethanol HCl in a 5% Tween suspension was administered by gavage to 10 male and 10 female Sprague-Dawley rats for 3 mo.[2] The dose was 56 mg/kg/d and was given at a volume of 10 ml/kg/d. A control group of 10 male and 10 female rats received vehicle alone. Clinical observations included a dull appearance of the fur and light brown areolas, fur being soiled with urine, and a brown discoloration of the urine. The results of histological and clinical examination of the treated animals were normal, with the exception of an increased serum glutamic-oxaloacetic transaminase (SGOT) activity and a slight increase in serum glutamic-pyruvic transaminase (SGPT) activity, alkaline phosphatase activity, and uric acid values. However, the SGPT, alkaline phosphatase, and uric acid values were within the normal limits. The significance of the increased SGOT value was relative in comparison to the control group, and it also did not exceed the normal limits of variation.

In a 13-wk study performed in accordance with OECD TG 408, groups of 10 male and 10 female Sprague-Dawley rats received 0, 4, 20, or 100 mg/kg/d 2,4-Diaminophenoxyethanol HCl (98.7% pure) in 5 ml purified water via gavage.[3,4] An additional 6 animals of each sex were added to the control and high-dose groups for a 4-wk recovery period after treatment, and an additional 6 animals were added to each group receiving the test material for toxicokinetic evaluation performed at

day 1 and during week 13 (results described above in the ADME section). Mortality determinations, clinical observations for toxicity (daily and weekly), functional test battery (during week 13), body weight and feed intake measurements (weekly), ocular examination, blood hematology and clinical chemistry, and urinalysis were all performed. At the end of the study, animals were killed and necropsied.

No treatment-related deaths, adverse clinical signs, or changes in feed intake were observed. In the high-dose group, increased salivation was observed. Body weights gains were decreased in the high-dose group during the dosing period, but returned to normal at the end of the recovery period. Urine discoloration, traces of glucose/nitrates, and bilirubin were observed in the high-dose group, but disappeared at the end of the recovery period. No increases in plasma glucose or bilirubin were observed. Isolated statistically significant organ weight differences were reported, including a 10% increase for relative brain weight in high-dose males and a 10% increase for relative kidney weight in high-dose females. After the recovery period, absolute (13%) and relative (15%) kidney weights and absolute (43%) and relative (52%) thymus weights were increased in females; these findings were not observed in males. No associated pathology was found in any of these organs. In the high-dose group, brown discoloration of thyroid glands and brown pigment observed on tissue examination were reported that persisted to the end of the recovery period. No inflammatory, degenerative, or proliferative changes were observed in the thyroid gland histology. Splenic hemosiderosis in the high-dose group that persisted to the end of the recovery period was reported, but there were no associated hematological changes. The no-observed-adverse-effect level (NOAEL) for this study was determined to be 20 mg/kg/d 2,4-Diaminophenoxyethanol HCl.[3,4]

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Dermal

In a dermal study, pregnant C5781/6 mice (crossed with T stock males) received 0.2 ml of 2,4-Diaminophenoxyethanol HCl dissolved in corn oil at concentrations of 15, 150, or 1500 mg/kg.[2] Test groups were comprised of 10 animals in the low- and mid-dose groups and 18 animals in the high-dose group. The solution was topically administered to a shaved area on the back of each mouse. Negative controls received corn oil only and positive controls received an intraperitoneal injection of benzo[α]pyrene on gestation day 10.5. No teratogenic effects or significant effects in skeletal development in the fetuses from the treated mice were observed when compared to the negative controls.

Oral

In a dose range-finding study, groups of 6 pregnant specific pathogen-free (SPF) rats received 0, 1, 25, 250, or 500 mg/kg/d 2,4-Diaminophenoxyethanol HCl via intragastric intubation on gestation days 1 to 10.[2] Control animals received the vehicle, distilled water, only. Clinical signs in the 250 and 500 mg/kg dose groups included increased post-dose salivation, elevated gait, stained coats, fur loss, and discolored urine. At the 125 mg/kg dose, the only signs observed were slight or moderate post-dose salivation and discolored urine. Feed consumption was reduced in the 250 and 500 mg/kg dose groups. Weight loss was observed in the high-dose group, reduced weight gain was observed in the mid-dose group, and minimal reduction of weight gain was observed in the low-dose group. Necropsy was performed after day 15 of gestation. With the exception of discolored fur in 4 animals, no compound-related changes were observed.

Based on the results of the dose-range-finding study, groups of 20 pregnant SPF rats received 0, 50, 100, or 200 mg/kg/d 2,4-Diaminophenoxyethanol HCl (10 ml/kg) by gavage on gestation days 6 to 15.[2] Controls animals received distilled water. Clinical signs included increased post-dose salivation and discolored urine in all dose groups and fur loss in the later stages of dosing and in the post-dose period in the 200 mg/kg dose group. Body weight gain was reduced at the 100 mg/kg dose and even more at the 200 mg/kg dose. On gestation day 20, the dams were killed, and the litters were examined. With the exception of discoloring of or loss of fur for some animals, no dose-related changes were observed at necropsy. No statistically significant changes in litter size were observed, and no statistically significant differences were observed in litter and fetal mean weight values. However, lower litter and fetal mean weight values in the 200 mg/kg dose group were considered to be treatment-related due to maternal effect and effects on skeletal development. In the 200 mg/kg dose groups, there was a significant dose-related increase in the incidence of skeletal anomalies and skeletal variants. This increase was thought to be related to a nonspecific retardation of embryo/fetal development during gestation. The incidence of major malformations and minor visceral anomalies was comparable among all groups.

The developmental toxicity potential of 2,4-Diaminophenoxyethanol HCl (98.7% pure) was assessed in female Sprague-Dawley rats on gestation days 6 – 19 in a study performed in accordance with OECD TG 414.[3,4] The rats received 0, 4, 20, or 125 mg/kg/d of the test material in purified water (dose volume 5 ml/kg) via gavage. Maternal clinical signs of toxicity, body weight, and feed intake were monitored. Dams were killed on gestation day 20. Gravid uterus weights were determined and fetuses were removed, sexed, weighed, and examined externally. Implantation sites, preimplantation loss, and live and dead fetuses were recorded. Half of the fetuses were examined for soft-tissue abnormalities and half for skeletal abnormalities.

Maternal results included excessive salivation and significantly decreased ($p < 0.001$) body weight gains in the high-dose group. Group mean feed consumption was statistically significantly reduced throughout the dosing period, when compared to the controls. There was no difference in the number of fetuses or in implantation sites between control and treatment groups. Only 1 dead fetus was reported in any group, and that was in the high-dose group (total fetuses = 311).

Fetal body weights were significantly decreased in male and female fetuses in the high-dose group. Short supernumerary 14th ribs, incomplete ossification of the centrum of the thoracic vertebrae, and incomplete ossification of the 5th sternebrae were reported in the high-dose group. External abnormalities were not seen in any fetus, except for 1 in the control group. No soft tissue malformations were observed in any group. The NOAEL for maternal and fetal developmental effects from 2,4-Diaminophenoxyethanol HCl was 20 mg/kg/d.[3,4]

GENOTOXICITY STUDIES

Genotoxicity was not observed in modified Ames tests using Escherichia coli, with or without metabolic activation, at concentrations of up to 2000 µg/plate 2,4-Diaminophenoxyethanol HCl.[2] *In several Ames tests with Salmonella typhimurium at concentrations of up to 1200 µg/plate 2,4-Diaminophenoxethanol HCl, with and without metabolic activation, no genotoxicity was observed. Exceptions were observed in a study where positive results were observed to S. typhimurium strains TA97, TA98, and TA1538 with the presence of at least 10% S9 metabolic activation with concentrations of 2,4-Diaminophenoxyethanol tested at 5 - 100 µg/plate, and in another study with strains TA98 and TA1538 where up to a 14-fold increase of revertants were observed at a concentration of 80 µg/ml (maximum concentration tested was 100 µg/ml) with 30% S9 metabolic activation. Positive results were also observed in a fluctuation test using S. typhimurium strains TA98 and TA1538, with and without metabolic activation (concentrations not reported). No genotoxicity was observed in studies of 2,4-Diaminophenoxyethanol HCl using several different strains of yeast, with and without metabolic activation, in forward mutation assays (up to 40 mM), gene conversion assays (up to 4000 µg/ml), and gene reversion tests (up to 6000 µg/ml). 2,4-Diaminophenoxyethanol HCl was not genotoxic in chromosomal aberration tests using Chinese hamster ovary (CHO) cells at concentrations of 0.6 or 1.2 mg/ml, with or without metabolic activation, or using human lymphocytes at concentrations of 10^{-5} to 10^{-3} M. Negative results were also observed in a forward mutation assay with Chinese hamster V79 cells exposed to up to 20 mM 2,4-Diaminophenoxyethanol HCl, with and without metabolic activation, in an unscheduled DNA synthesis test with HeLa cells exposed to up to 0.2 mM 2,4-Diaminophenoxyethanol HCl.*

The urine of male CD-1 mice that received 2,4-Diaminophenoxyethanol HCl (in corn oil; up to 1500 mg/kg) dermally for 3 d was not mutagenic in an Ames test.[2] *No mutagenicity was observed in a similar study in the urine of male Wistar rats that received 10 mg/kg 2,4-Diaminophenoxyethanol HCl orally in distilled water, dermal applications of 120 mg 2,4-Diaminonphenoxyethanol HCl in 4 ml phosphate buffer, or intraperitoneally received 100 mg/kg of the test material in 0.9% sodium chloride.*

No genotoxicity was observed to 2,4-Diaminophenoxyethanol HCl in a mouse dominant-lethal assay (dermal applications of up to 1500 mg/kg) or in a mouse spot test for somatic mutations (dermal applications of up to 1500 mg/kg). In a mouse micronucleus test, no increase in micronucleated cells were observed when the animals received up to 100 µg/ml 2,4-Diaminophenoxyethanol HCl; however, the ratio of normochromatic to polychromatic erythrocytes was significantly reduced.

In vitro and in vivo genotoxicity studies on 2,4-Diaminophenoxyethanol HCl summarized here are detailed in Table 3. In an Ames test of up to 5000 µg/plate, 2,4-Diaminophenoxyethanol HCl was genotoxic in strain TA98 with metabolic activation, but these results were not observed without activation or in the other *S. typhimurium* strains tested, with or without activation.[3,4] Genotoxicity to 2,4-Diaminophenoxyethanol HCl was also observed in a micronucleus assay (up to 2410 µg/ml) and chromosome aberration test (up to 2200 µg/ml); both tests used human lymphocytes, with and without metabolic activation. 2,4-Diaminophenoxyethanol HCl was not genotoxic in a mammalian cell gene mutation test in L5178Y mouse lymphoma cells at up to 2410 µg/ml. In rat studies, genotoxicity was not observed with 2,4-Diaminophenoxyethanol HCl at up to 1500 mg/kg in a mammalian bone marrow micronucleus or in an unscheduled DNA synthesis assay.

CARCINOGENICITY STUDIES

Oral

In oral carcinogenicity studies, groups of 50 male and 50 female BDF_1 mice received 0, 0.004 or 0.007% 2,4-Diaminophenoxyethanol HCl in tap water for 104 wk.[2] *In a similar study, groups of 50 male and 50 female F344 rats received 0, 0.05 and 0.1% 2,4-Diaminophenoxyethanol HCl in tap water for the same duration of time. No adverse effects were reported during treatment of the mice; however, marked decreases in weight gain were observed in the 0.1% dose group male and female rats when compared to controls. Mean body weight gains for both treated groups of male and female rats were reduced when compared to the controls. At necropsy, pigment deposits were observed in the epithelial cells of thyroid follicles in both treated groups of mice and in the 0.1% dose group of rats. Deposits were histochemically negative for silver and iron and were unrelated to tumor incidence. No carcinogenic effects were reported in either species of rodent.*

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

A 4% solution of 2,4-Diaminophenoxethanol HCl in distilled water (5 ml, pH 8.5) was applied to shave intact and abraded skin of 6 albino Bouscat rabbits (3 males, 3 females).[2] *The solution had a primary irritation index of 0.8/8 and was determined to be slightly irritating.*

In a dermal irritation study performed in accordance with OECD TG 404, 3 female New Zealand White rabbits received 0.5 g of 2,4-Diaminophenoxyethanol HCl (99.4% pure) on a clipped area (6 cm^2) of the right flank.[3,4] The test material was moistened with 0.5 ml of paraffin oil. A non-occlusive dressing was applied for 4 h and then removed. Cutaneous reactions were assessed on the treated and control sites (untreated left flank) at 1, 24, 48, and 72 h after the dressing was removed. Slight erythema was observed in 1 animal at the treatment site at the 48-h observation. No other effects were observed and the material, as tested, was not considered a dermal irritant in this study.

Sensitization

Animal

In a modified guinea pig maximization test, 10 female Hartley guinea pigs received two 0.2 ml intradermal injections of 50% Fruend's adjuvant prior to dermal application of 2,4-Diaminophenoxyethanol HCl moistened with distilled water to a 3 cm^2 area of deeply abraded skin.[2] *The test site was occluded for 48 h. A second dermal application of 25% test material in petroleum jelly was administered on day 7 and also occluded for 48 h. A challenge patch with 25% 2,4-Diaminophenoxy-ethanol HCl in petroleum jelly was applied on day 21 to a 5 cm^2 area of shaved, untreated skin. The test site was occluded for 24 h. An additional 5 non-sensitized guinea pigs were also treated with 25% test material to serve as the control group. Readings were taken at 48 and 72 h after challenge application. Erythema was observed in 3 of the 10 animals, all of which resolved within 5 d. It was determined that 2,4-Diaminophenoxyethanol HCl had a low sensitizing potential in humans.*

In a local lymph node assay (LLNA) performed in accordance with OECD TG 429, groups of 4 female CBA/J mice received 0.5, 1, 2.5, 5, or 10% (w/v) 2,4-Diaminophenoxyethanol HCl (98.7% pure) in dimethyl sulfoxide (DMSO).[3,4] A negative control group received DMSO alone and a positive control group received 25%(w/v) α-hexylcinnamaldehyde in DMSO. A volume of 25 µl of test material was applied to each mouse ear for 3 consecutive days. Ear thickness was measured on days 1, 2, 3, and 6. On day 6, a single injection of ^{3}H-methyl thymidine was given to the mice. After 5 h, the animals were killed, the auricular lymph nodes were excised and pooled for each test group, and radioactivity was measured. Treatment-related, but not dose-related, ear swelling was observed. The stimulation index (SI) was 0.92, 1.56, and 1.17 for the 0.5, 1, and 2.5% dose groups, respectively. Positive lymphoproliferative responses were observed in the 5% group (SI = 4.21) and the 10% group (SI = 7.42). The positive control had an SI of 8.51. The estimated concentrations of an SI of 3 (EC_3) value was determined to be 3.2%, indicating that 2.4-Diaminophenoxyethanol HCl is a moderate skin sensitizer.

A guinea pig sensitization study on 2,4-Diaminophenoxyethanol HCl (99.6% pure) was performed using 10 male and 10 female Dunkin-Hartley guinea pigs.[3,4] This study was performed in accordance with OECD TG 406. Animals were clipped and/or shaved on the anterior left flank before each application. The animals received 3 induction exposures with a gauze pad (~ 4 cm^2) containing 500 mg neat 2,4-Diaminophenoxyethanol HCl. Control animals (n = 5 of each sex) received gauze pads with 0.5 ml water. The pads were occluded for 6 h and test sites were evaluated at 24 h after dressing removal. Induction applications occurred on days 1, 8, and 15 of the study. On day 29, control and treated animals received a topical challenge of 0.5 ml water applied to the left flank and 500 mg of the test material to a naïve site on the right flank. Sites were assessed at 24, 48, and 72 h post-challenge. The test material colored the skin purple. Slight erythema was observed in one treated animal of each sex at the 48-h observation period only. It was concluded that 2,4-Diaminophenoxyethanol HCl was not sensitizing.

OCULAR IRRITATION STUDIES

Animal

In an ocular irritation study, 0.1 ml of a 4% aqueous solution of 2,4-Diaminophenoxyethanol HCl was instilled in the conjunctival sac of one eye of each of 6 albino Bouscat rabbits (3 males, 3 females).[2] *The other eye was not treated and served as a control. Eyes were not rinsed following application of the test material. The ocular irritation index was estimated to be 1.66/110 after 24 h, 0.331/110 after 48 h, and 0/110 after 72 h, and 4 and 7 d. The test material was determined to be practically not irritating.*

2,4-Diaminophenoxyethanol HCl (100 mg; 99.4% pure) was instilled neat into the conjunctival sac of the left eye of 3 female New Zealand White rabbits in an ocular irritation study performed in accordance with OECD TG 405.[3,4] The eyes were not rinsed and the right eye served as the control. Ocular effects were evaluated at 1, 24, 48, and 72 h post-exposure and at days 8 and 15. Moderate to marked chemosis, slight to moderate conjunctival redness, slight to moderate corneal opacification, and slight iridal lesions were observed. While lessened, these effects had not disappeared after 15 d. 2,4-Diaminophenoxyethanol HCl was considered an ocular irritant in this study.

In another ocular irritation study performed in accordance with OECD TG 405, 3 female New Zealand White rabbits received instillations of 0.1 ml of a 4% dilution of 2,4-Diaminophenoxyethanol HCl (98.7% pure) in purified water in the conjunctival sac of the left eye.[3,4] The eyes were not rinsed, and the right eye served as the control. Ocular effects were evaluated at 1, 24, 48, and 72 h post-exposure. Slight chemosis and redness were observed in 2/3 animals in the treated eyes, but the effects did not persist beyond day 2. There were no corneal or iridal effects. 2,4-Diaminophenoxyethanol HCl at a 4% dilution in water was not considered an ocular irritant.

CLINICAL STUDIES

Case Reports

A 43-yr-old female reported dermatitis of the scalp and neck after using a hair coloring product every 6 wk.[14] In one incidence, the application of the hair dye provoked an immediate pruritis of the scalp and a malaise that lasted for 15 min. The subsequent application resulted in a severe reaction with generalized pruritus and erythema, dyspnea, vomiting, and hypotonia. The symptoms disappeared after 2 h. Contact dermatitis appeared thereafter and lasted for 8 d. When skin tests (open test) were performed, oxidized 2,4-Diaminophenoxyethanol HCl yielded a positive result after 20 min. Further patch testing yielded ++ positive reactions for p-phenylenediamine base (1% pet) and toluene-2,5-diamine (1% pet).

MARGIN OF EXPOSURE

MOE is a quantitative factor calculated for cosmetic ingredients by dividing the NOAEL obtained for an ingredient in an animal experiment by the estimated systemic exposure dose (SED) for the ingredient in humans, generally according to US Environmental Protection Agency (EPA) and European Commission SCCS guidelines. An MOE value greater than 100 has traditionally been considered an indication of safety. The MOE is sometimes referred to as margin of safety (MOS), despite the parameters being definitionally different.

The SCCS calculated an MOE value for 2% 2,4-Diaminophenoxyethanol HCl as used under oxidative conditions to be 500.[5] (The maximum use concentration reported to the Panel is 0.56 - 2.4% in hair dyes).[10] This calculation is based on the NOAEL of 20 mg/kg bw/d (from a 13-wk oral rat study) and a SED of 0.04 mg/kg bw (skin area surface of 580 cm^2 x absorption through skin of 4.33 $\mu g/cm^2$ x 0.001 (unit conversion)/typical human bw of 60 kg).

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. 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are reported to be used in oxidative hair dye formulations. While the safety of individual hair dye ingredients is not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer. A detailed summary of the available hair dye epidemiology data is available at https://www.cir-safety.org/cir-findings.

SUMMARY

2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are reported to function as hair colorants in cosmetics, according to the *Dictionary*. These ingredients are oxidative dyes used in permanent hair dye formulations.

According to 2023 VCRP survey data, 2,4-Diaminophenoxyethanol HCl is reported to be used in 93 formulations (89 of which are in hair coloring preparations and 4 are in eye makeup preparations) and 2,4-Diaminophenoxyethanol Sulfate is reported to be used in 10 formulations (9 of which are in hair coloring preparations and 1 is in an eye makeup preparation). The frequencies of use for these ingredients have only slightly changed since last reviewed by the Panel. In 2006, 2,4-Diaminophenoxyethanol HCl was reported to be used in 115 formulations and 2,4-Diaminophenoxyethanol Sulfate was reported to be used in 5 formulations; all uses for these ingredients were in hair dyes and coloring products. The results of the concentration of use survey conducted by the Council in 2022 indicate 2,4-Diaminophenoxyethanol HCl has a maximum concentration of use range of 0.56 - 2.4% in hair dyes. 2,4-Diaminophenoxyethanol Sulfate has a maximum concentration of use range of 0.25 - 0.35% in hair dyes. In the 2007 amended report, the maximum concentration of uses range for 2,4-Diaminophenoxyethanol HCl was 0.05 - 2% in hair dyes; the sulfate salt was reported to be used at a maximum concentration of 0.4 - 2% in hair dyes (2% is the final on-head concentration after mixing with hydrogen peroxide for both ingredients).

With regard to the reported use in eye makeup preparations, the US Federal FD&C Act mandates that color additives must be approved by FDA for their intended use before they are used. 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are not approved color additives in non-hair dye cosmetic products, and thereby, used in eye makeup products is not permitted. Furthermore, the FD&C Act also specifies that coal tar hair dyes must not be used for dyeing the eyelashes or eyebrows; to do so may cause blindness.

Under European regulations for cosmetic ingredients, 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are listed in Annex III with the restrictions that these ingredients may be used as hair dyes substances in oxidative hair dye products. Additionally, these ingredients may be used in products intended for coloring eyelashes. The SCCS has determined that the use of 2,4-Diaminophenoxyethanol HCl or 2,4-Diamionphenoxyethanol Sulfate in oxidative hair dye formulations at a maximum final concentration of 2.0% (after mixing with hydrogen peroxide) does not pose a risk to the health of the consumer, apart from its sensitizing potential.

In a percutaneous absorption study, the dermal delivery of 2.0% 2,4-Diaminophenoxyethanol HCl through human dermatomed skin was reported to be 1.74 µg equivalents/cm^2 (0.41% of the applied dose) for the oxidative formulation and 6.55 µg equivalents/cm^2 (1.68% of the applied dose) for the non-oxidative formulation. As part of a 13-wk subchronic toxicity study, rats received 4, 20, or 100 mg/kg/d 2,4-Diaminophenoxyethanol HCl in purified water via gavage for toxicokinetic evaluation performed on day 1 and during week 13. Plasma levels of 2,4-diaminophenoxyethanol were not detectable in the low-dose group. In the 20 mg/kg/d group, plasma levels of 2,4-diaminophenoxyethanol were detectable at 30 min after dosing (no shorter determination was made). In the 100 mg/kg/d dose group, plasma levels of 2,4-diaminophenoxyethanol were maximal at 30 min. Thereafter, the plasma levels decreased steadily until the last quantifiable time-point (2 h on day 1, 8 h in week 13).

In an acute oral toxicity study in rats, the LD_{50} for 2,4-Diaminophenoxyethanol HCl was close to 1000 mg/kg, which was the only dose tested. During the study, 1 male rat and 3 female rats were found dead. Clinical signs of toxicity included hypoactivity, piloerection, lateral decubitus, and tonic-clonic convulsions. No abnormalities were observed during necropsy.

The NOAEL for a 13-wk gavage study in rats was 20 mg/kg/d 2,4-Diaminophenoxyethanol HCl (dose concentrations tested were 0, 4, 20, or 100 mg/kg/d). No treatment-related deaths, adverse clinical signs, or changes in feed intake were observed. Body weight gains were decreased in the high-dose group during the dosing period, but returned to normal at the end of the recovery period. Urine discoloration, traces of glucose/nitrates, and bilirubin were observed in the high-dose group, but disappeared at the end of the recovery period. Isolated statistically significant organ weight differences were reported, including 10% increase for relative brain weight in high-dose males and 10% increase for relative kidney weight in high-dose females. After the recovery period, absolute and relative kidney weights and absolute and relative thymus weights were increased in females; these findings were not observed in males. In the high-dose group, brown discoloration of thyroid glands and brown pigment observed on tissue examination were reported that persisted to the end of the recovery period. Splenic hemosiderosis in the high-dose group that persisted to the end of the recovery period was reported, but there were no associated hematological changes.

In a rat developmental toxicity study, the NOAELs for maternal and fetal effects from 2,4-Diaminophenoxyethanol HCl were 20 mg/kg/d (dose concentrations tested were 0, 4, 20, or 125 mg/kg/d). Maternal results included excessive salivation and significantly decreased ($p < 0.001$) body weight gains in the high-dose group. Group mean feed consumption was statistically significantly reduced throughout the dosing period, when compared to the controls. Fetal body weights were significantly decreased in male and female fetuses in the high-dose group. Short supernumerary 14^{th} ribs, incomplete ossification of the centrum of the thoracic vertebrae, and incomplete ossification of the 5th sternebrae were reported in the high-dose group.

In an Ames test of up to 5000 µg/plate, 2,4-Diaminophenoxyethanol HCl was genotoxic in strain TA98 with metabolic activation, but these results were not observed without activation or in the other *S. typhimurium* strains tested, with or without activation. Genotoxicity to 2,4-Diaminophenoxyethanol HCl was also observed in a micronucleus assay (up to 2410 µg/ml) and chromosome aberration test (up to 2200 µg/ml), both using human lymphocytes and performed with and without metabolic activation. 2,4-Diaminophenoxyethanol HCl was not genotoxic in a mammalian cell gene mutation test in L5178Y mouse lymphoma cells at up to 2410 µg/ml. In rat studies, genotoxicity was not observed with 2,4-Diaminophenoxyethanol HCl at up to 1500 mg/kg in a mammalian bone marrow micronucleus or in an unscheduled DNA synthesis assay.

In a dermal irritation study, 99.4% pure 2,4-Diaminophenoxyethanol HCl was not considered a dermal irritant to rabbit skin. 2,4-Diaminophenoxyethanol HCl (tested at up to 10%) was a moderate skin sensitizer in an LLNA with an EC_3 value of 3.2%; however, 2,4-Diaminophenoxyethanol HCl (99.6%) was not sensitizing in a guinea pig sensitization study when tested neat. In ocular irritation studies, 2,4-Diaminophenoxyethanol HCl was an ocular irritant in rabbit eyes when tested neat (99.4% pure), but it was not considered an ocular irritant at a 4% dilution in water. A case study of a severe reaction to a hair dye containing 2,4-Diaminophenoxyethanol HCl has been reported.

The SCCS calculated an MOE value for 2% 2,4-Diaminophenoxyethanol HCl as used under oxidative conditions to be 500. This calculation is based on the NOAEL of 20 mg/kg bw/d (from a 13-wk oral rat study) and an SED of 0.04 mg/kg bw (skin area surface of 580 cm^2 x absorption through skin of 4.33 µg/cm^2 x 0.001 (unit conversion)/typical human bw of 60 kg). The MOE value is greater than 100, a figure generally accepted as the threshold for considering an ingredient safe for use.

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.

1991 ORIGINAL REPORT DISCUSSION

2,4-Diaminophenoxyethanol HCl was slightly irritating to the skin of rabbits.[2] *In a sensitization study using guinea pigs, erythema was observed in 3 of 10 guinea pigs, all of which recovered in 5 d. Based on the results of this study, investigators suggested that 2,4-Diaminophenoxyethanol HCl could have a low sensitizing potential in humans. The Expert Panel used these results, due to a lack of human data, in its safety assessment of 2,4-Diaminophenoxyethanol HCl. Hair dyes containing 2,4-Diaminophenoxyethanol HCl are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal FD&C Act of 1938 when cautionary statements and patch test instructions are conspicuously displayed on the labels. Prophetic patch testing of hair dye formulations with open patches in less predictive of skin reactions than patch testing with closed patches. False negative reactions may occur. Some persons may be sensitized, even under the proper conditions of use.*

2007 AMENDED REPORT DISCUSSION

The Panel considered that the available acute and subchronic oral, ocular, and dermal toxicity data are adequate to support the safety of 2,4-Diaminophenoxyethanol HCl with respect to systemic toxicity endpoints.[3] *This ingredient did not produce significant toxicity to the reproductive system or affect development of fetuses in animal studies at levels that were not maternally toxic. Based on the low dermal absorption of 2,4-Diaminophenoxyethanol HCl from oxidative hair dye formulations, such maternally toxic levels are highly unlikely. The Expert Panel noted that there were mixed results in the available genotoxicity data; however, 2,4-Diaminophenoxyethanol was not carcinogenic in mouse and rat studies.*

2,4-Diaminophenoxyethanol HCl was slightly to non-irritating to the skin of rabbits. Dermal sensitization study results are mixed, but a maximization study and a local lymph node assay were positive, so this ingredient could be considered a moderate skin sensitizer. It is relevant that hair dyes containing these ingredients, as coal tar hair dye products, are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal FD&C Act, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Expert Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposure.

No toxicity studies were identified specifically for the sulfate salt, 2,4-Diaminophenoxyethanol Sulfate, in the published literature. The toxicities of the two salts are expected to be the same, and their maximum use concentrations are the same, so exposures as used in hair dyes would be the same. Therefore, the Expert Panel determined that the toxicity data on 2,4-Diaminophenoxyethanol HCl could be extrapolated to 2,4-Diaminophenoxyethanol Sulfate.

While there were no human studies that specifically addressed these two ingredients, the Panel did review the available human epidemiology data. In considering these data, the Panel concluded that the available hair dye epidemiology studies are insufficient to conclude there is a casual relationship between hair dye use and cancer and other endpoints, based on lack of strength of the associations and inconsistency of findings. Use of direct hair dyes, while not the focus of all investigations, appears to have little evidence of any association with adverse events as reported in epidemiology studies.[3]

DISCUSSION

To be developed…

CONCLUSION

To be determined…

TABLES

Table 1. Chemical properties

Property	Value	Reference
	2,4-Diaminophenoxyehanol HCl	
Physical Form	white powder, slightly gray	2
	lavender-gray powder	2
	light grey to light pink powder	4
Formula Weight (g/mol)	241	2
Density (g/ml @ 20 ºC)	1.409 (estimated)	6
Vapor pressure (mm Hg @ 20 ºC)	0 (estimated)	6
Melting Point (ºC)	198-216	2
	84 (free base)	2
	242.5	4
Water Solubility (@ 25 ºC)	soluble	2
(g/l @ 20 ºC)	425	4
Other Solubility (@ 25 ºC)	insoluble in 95% ethanol; acetone	2
(g/100 ml)	≤ 1 in ethanol; > 10 in DMSO	4
log P_{ow} (@ 20 ºC)	0.99	4,5
UV/Visible Spectrum (λ_{max}; nm)	238, 286	2
	2,4-Diaminophenoxyethanol Sulfate	
Physical Form	white powder	4
Formula Weight (g/mol)	266.27	4
	302.3 (dihydrate)	5
Density (g/ml @ 20 ºC)	1.506 (estimated)	7
Vapor pressure (mm Hg @ 20 ºC)	0 (estimated)	7
Water Solubility (g/l @ pH 1.9)	50-100 (dihydrate)	5
(g/l @ 20 ºC, pH 2.1)	98.2	7
Other Solubility (g/100 ml)	< 10 in ethanol; > 100 in DMSO	5
log P_{ow}	-0.612 (dihydrate)	5

Table 2. Frequency (2023/2006) and concentration (2022/2007) of use according to likely duration and exposure and by product category

	2,4-Diaminophenoxyethanol HCl				2,4-Diaminophenoxyethanol Sulfate			
	# of Uses		*Max Conc of Use (%)*		*# of Uses*		*Max Conc of Use (%)*	
	2023[9]	**2006**[3]	**2022**[10]	**2007**[3]	**2023**[9]	**2006**[3]	**2022**[10]	**2007**[3]
Totals*	**93**	**115**	**0.00066-2.4**	**0.05-2**†	**10**	**5**	**0.25-0.35**	**0.4-2**†
summarized by likely duration and exposure**								
Duration of Use								
Leave-On	*4*	*NR*	*NR*	*NR*	*1*	*NR*	*NR*	*NR*
Rinse-Off	*89*	*115*	*0.00066-2.4*	*0.05-2*	*9*	*5*	*0.25-0.35*	*0.4-2*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	4	NR	NR	NR	1	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	4	NR	NR	NR	1	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Hair-Coloring	89	NR	0.00066-2.4	0.05-2	9	5	0.25-0.35	0.4-2
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
as reported by product category								
Eye Makeup Preparations								
Eyeliner	1	NR	NR	NR				
Other Eye Makeup Preparations	3	NR	NR	NR	1	NR	NR	NR
Hair Coloring Preparations								
Hair Dyes and Colors (all types requiring caution statements and patch tests)	82	115	0.56-2.4	0.05-2	9	5	0.25-0.35	0.4-2
Hair Tints	3	NR	NR	NR				
Hair Shampoos (coloring)	3	NR	NR	NR				
Hair Lighteners with Color	NR	NR	0.02	NR				
Hair Bleaches	NR	NR	0.00066	NR				
Other Hair Coloring Preparation	1	NR	NR	NR				

NR – not reported

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

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

† Maximum on head concentration (4%) reduced to a final on-head concentration of 2% after mixing with hydrogen peroxide upon application

Table 3. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
			IN VITRO			
2,4-Diaminophenoxyethanol HCl, > 99.5% pure	1.3 - 5000 µg/plate	not reported	*Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	Bacterial reverse mutation test in accordance with OECD TG 471; with and without S9 metabolic activation; appropriate positive controls used	Genotoxic in strain TA98 with metabolic activation; genotoxicity was not noted in the other strains; positive controls yielded expected results	[3,4]
2,4-Diaminophenoxyethanol HCl, > 99.5% pure	85 - 2410 µg/ml	not reported	human lymphocytes	Micronucleus assay; cultures exposed to mitogen stimulation 24 or 48 h prior to treatment; cultures exposed to test material for 20 h without metabolic activation and for 3 h with metabolic activation; appropriate positive and negative controls used	Genotoxic; test material-induced micronuclei after 48 h mitogen stimulation, with and without metabolic activation; no increases in micronuclei were observed in cultures exposed to mitogen stimulation for 24 h prior to treatment, with or without metabolic activation	[3,4]
2,4-Diaminophenoxyethanol HCl, 98.7% pure	50 - 2200 µg/ml	not reported	human lymphocytes	Chromosome aberration test in accordance with OECD TG 473; appropriate positive controls used; in experiment 1, cultures were exposed to test material for 3 h with and without metabolic activation; in experiment 2, cultures with metabolic activation were exposed for 3 h and cultures without metabolic activation were exposed for 20 h	Genotoxic with and without metabolic activation; highest concentration in experiment 1 (1542 µg/ml) induced approximately 54 and 55% mitotic inhabitation without and with metabolic activation, respectively; in experiment 2, highest concentrations without metabolic activation (120.9 µg/ml) and with metabolic activation (2200 µg/ml) induced approximately 66 and 39% mitotic inhibition, respectively; increased induction ($p < 0.001$) of chromosome aberrations observed following 3-h exposure with metabolic activation and following 20-h exposure without metabolic activation	[3,4]
2,4-Diaminophenoxyethanol HCl; > 99.5% pure	200 – 2410 µg/ml	not reported	L5178Y mouse lymphoma cells	Mammalian cell gene mutation test at the *hprt* locus in accordance with OECD TG 476; 3 h exposure with and without metabolic activation; appropriate negative and positive controls used	Not genotoxic; no statistically significant increases in mutant frequency were observed without metabolic activation; with metabolic activation, a small but statistically significant increase in mutant frequency were observed at 1000 and 1600 µg/ml in experiment 1 and at 400, 1600, and 2410 µg/ml in experiment 2, however, there was no evidence of a statistically significant linear trend in experiment 1 and only a weak linear trend in experiment 2; all mutant frequencies observed in the treated cultures fell within historical negative control range	[3,4]

Table 3. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
			IN VIVO			
2,4-Diaminophenoxyethanol HCl, > 99.5% pure	375 - 1500 mg/kg	water	groups of 5 male and 5 female Sprague-Dawley rats	Mammalian bone marrow micronucleus test in accordance with OECD TG 474; rats received a single dose of test material via gavage; rats were killed 24 h after treatment, an additional high-dose group of rats were killed 48 h after treatment; appropriate positive and negative controls used	Not genotoxic; one animal in 1500 mg/kg group died (sex not stated); no statistically significant increase in micronucleus frequencies were observed in polychromatic erythrocytes from male or female rats at any dose tested; 1 male (48-h kill) and 1 female (24-h kill) in the high-dose level had elevated micronucleus responses, but overall micronucleus responses were not statistically significant when compared to vehicle control and isolated increases were considered not biologically-relevant; no statistically significant decreases in polychromatic erythrocyte: normochromatic erythrocyte ratios observed in doses up to 1500 mg/kg in either sex	[3,4]
2,4-Diaminophenoxyethanol HCl, > 99.5% pure	375 - 1500 mg/kg	not reported	groups of 4 or 6 male Sprague-Dawley rats	Unscheduled DNA synthesis assay in accordance with OECD TG 494; rats received a single dose of test material via gavage; animals were killed after 2 - 4 h or 14 -16 h; appropriate positive and negative controls used	Not genotoxic; in animals that received 375 mg/kg test material at the 14 - 16 h kill, high percentages of nuclei with < 5 net nuclear grains occurred in 2 out of 3 animals, and one of the responding animals had a response in only 1 out of 3 slides; in the other treated groups, the unscheduled DNA synthesis response was similar to the vehicle controls for both harvest times; the observed effects in the 375 mg/kg dose group were considered not treatment-related	[3,4]

REFERENCES

1. Nikitakis J, Kowcz A. 2024. Web-Based *International Cosmetic Ingredient Dictionary and Handbook*. https://incipedia.personalcarecouncil.org/winci/. Last Updated: 2024.
2. Elder R(ed.). Final Report on the Safety Assessment of 2,4-Diaminophenoxyethanol Dihydrochloride. *J Am Coll Toxicol*. 1991;10(1):113–134.
3. Burnett CL, Bergfeld WF, Belsito DV, et al. 2007. Amended Safety Assessment of 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate. https://cir-reports.cir-safety.org/view-attachment?id=4d65c18f-8c74-ec11-8943-0022482f06a6. Last Updated: 2007.
4. Scientific Committee on Consumer Products. 2006. Opinion on 2,4-Diaminophenoxyethanol and its salts. COLIPA No. A42. SCCP/0979/06. https://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_042.pdf. Last Updated: 2006.
5. Scientific Committee on Consumer Safety. 2010. Opinion on 2,4-Diaminophenoxyethanol dihydrochloride and sulfate. COLIPA No. A042. SCCS/1367/10. https://health.ec.europa.eu/document/download/86a2e0d7-3175-4f5f-a7a4-b0e9df0dc173_en?filename=sccs_o_035.pdf. Last Updated: 2010.
6. European Chemicals Agency. 2021. 2-(2,4-diaminophenoxy)ethanol dihydrochloride. https://chem.echa.europa.eu/100.060.306/dossier-view/0ff4de6f-12d2-4d20-8ec2-27230eaa4474/338dbed2-3ce5-41ab-8256-998babf968c1_338dbed2-3ce5-41ab-8256-998babf968c1?searchText=66422-95-5. Last Updated: 2021. Date Accessed: July 19, 2024.
7. European Chemicals Agency. 2021. [4-(2-hydroxyethoxy)-1,3-phenylene]diammonium sulphate. https://chem.echa.europa.eu/100.067.900/dossier-view/fe016723-03ac-4bf8-ae53-6bf22d16dc7a/08aa9fcd-dda6-46e8-b583-22a93976608f_08aa9fcd-dda6-46e8-b583-22a93976608f?searchText=70643-20-8. Last Updated: 2021. Date Accessed: July 19, 2024.
8. Nikitakis J, Kowcz A. 2024. Web-Based International Cosmetic Ingredient Dictionary and Handbook. https://incipedia.personalcarecouncil.org/winci/. Last Updated: 2024.
9. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). 2023. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients.College Park, MD. [Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2023; received February 2, 2023].
10. Personal Care Products Council. 2022. Concentration of Use by FDA Product Category: 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate. [Unpublished data submitted by the Personal Care Products Council on October 28, 2022].
11. Goossens A. Self-testing for contact sensitization to hair dyes. *Contact dermatitis*. 2012;66(6):299.
12. Thyssen JP, Sosted H, Uter W, et al. Self-testing for contact sensitization to hair dyes - scientific considerations and clinical concerns of an industry-led screening programme. *Contact dermatitis*. 2012;66(6):300.
13. EUR-Lex. 2009. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast). (Text with EEA relevance). https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02009R1223-20231201&qid=1704209440026. Last Updated: 2009.
14. Nosbaum A, Dupin C, Nicolas J, Berard F. Severe immediate hypersensitivity and allergic contact dermatitis caused by hair dyes. *Contact Dermatitis*. 2012;67(1):52–53.

JOURNAL OF THE AMERICAN COLLEGE OF TOXICOLOGY
Volume 10, Number 1, 1991
Mary Ann Liebert, Inc., Publishers

10
Final Report on the Safety Assessment of 2,4-Diaminophenoxyethanol Dihydrochloride

2,4-Diaminophenoxyethanol Dihydrochloride (2,4-DAPE) is an aromatic amine that is used as a coupler in permanent (oxidative) hair dyes at concentrations up to 1.0%.

2,4-DAPE was slightly toxic in rats and mice. No significant adverse changes were observed in a subchronic toxicity test. 2,4-DAPE was practically nonirritating when a 4% aqueous solution was instilled into the conjunctival sacs of the eyes of rabbits. 2,4-DAPE was slightly irritating to the skin of rabbits when tested at a concentration of 4.0%.

Based on animal studies, 2,4-DAPE was judged to produce low level sensitization in humans. Coal tar hair dyes, including those containing 2,4-DAPE, are exempt from the principal adulteration provision and the color additive provisions in Sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation.

No teratogenic effects were observed due to administration of 2,4-DAPE. No mutagenic activity attributable to 2,4-DAPE was observed in 22 mutagenic studies. However, in a few studies, a marginal mutagenic response was reported.

In two oral carcinogenic studies, one in which mice were dosed with 0.04 and 0.07% 2,4-DAPE and the second in which rats were dosed with 0.05 and 1.0% 2,4-DAPE, no carcinogenic effects were produced.

On the basis of the data presented in this report, it is concluded that 2,4-Diaminophenoxyethanol Dihydrochloride is safe as a cosmetic ingredient in the present practices of use and concentration.

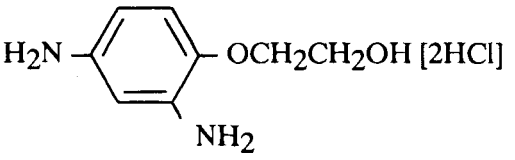
INTRODUCTION

2,4-Diaminophenoxyethanol Dihydrochloride is an aromatic amine used as a coupler mixed with primary intermediates in permanent (oxidative) hair dyes.[1]

CHEMISTRY

Definition and Structure

2,4-Diaminophenoxyethanol Dihydrochloride (CAS No. 66422-95-5) is the aromatic amine salt which conforms to the formula:[2]



2,4-Diaminophenoxyethanol Dihydrochloride

2,4-Diaminophenoxyethanol, Dihydrochloride (2,4-DAPE) is also know as 1-β-Hydroxyethyloxy-2,4-diamino-benzene, Dihydrochloride, (Diamino-2',4'-phenoxy)-2-ethanol, Dichlorhydrate, 2-(2',4'-Diaminophenoxy)ethanol, Dihydrochloride,[1] 1-(2-Hydroxyethyloxy)-2,4-diamino-benzene,[3] and Ethanol, 2-(2,4-Diaminophenoxy)-, Dihydrochloride.[2]

Properties

2,4-DAPE is an odorless white, slightly gray powder that is soluble in water and insoluble in acetone and 95% ethanol.[3] Physical and chemical properties of 2,4-DAPE are summarized in Table 1.

Impurities

1,3-Diaminobenzene, 2,4-diamino-1-methoxybenzene, and 2,4-diamino-1-ethoxy-benzene are not detected as impurities in 2,4-DAPE.[3]

TABLE 1. PROPERTIES OF 2,4-DIAMINOPHENOXYETHANOL HCl

Property	*Description*	*Reference*
Physical appearance	White powder, slightly gray	3
	Lavender-gray powder	4
Odor	None	3
Molecular weight	241	1
Empirical formula	$C_8H_{12}N_2O_2$ [2HCl]	2
Melting point	198-216°C	5
Free base		
Melting point	84°C	6
Solubility at 25°C	Water soluble	3
	95% ethanol insoluble	3
	Acetone insoluble	3
Spectrum absorbance	238 ± 5 - 0.630 ± 0.050	3
(λm - Absorbancy)	286 ± 5 - 0.260 ± 0.020	3
Decomposition point	198-217°C	1
Thermopan microscope		
Titer, potentiometry	≥ 99.5%	1
Assay, % Acid function	99 ± 1	3
Chloride concentration	28.0 - 31.0	3

USE

Cosmetic Use

The product formulation data submitted to the Food and Drug Administration[7] for 2,4-DAPE indicated that it is contained in 82 hair dye and coloring products at a concentration of ≤ 5%. Seventy-six of the formulations occur at a concentration of ≤ 0.1% (Table 2).

The FDA cosmetic product formulation computer printout[7] is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations.[8] Ingredients are listed in preset concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product. The actual concentration would be a fraction of that reported to the FDA. Data submitted within the framework of preset concentration ranges provide the opportunity 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.

When the amount of 2,4-DAPE added to an oxidative hair dye formulation is based on a desired shade, the concentrations are generally as follows: less than 0.025% in light tones, between 0.025 and 0.1% in medium tones, and between 0.1% and 4% in darker tones (which represent a smaller part of the market).[1] The maximum 4% concentration allowed for use is reduced to a final on-head concentration of 2% when combined with hydrogen peroxide on application.

The hair dyes containing 2,4-DAPE, as coal tar hair dye products, are exempt from the principal adulteration provision and from the color additive provision in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when their label bears a caution statement as well as patch test instructions to determine whether the product causes skin irritation.[9] The following caution statement should 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.

TABLE 2. PRODUCT FORMULATION DATA FOR 2,4-DIAMINOPHENOXYETHANOL HCl[7]

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (%)*		
			>1-5	*>0.1-1*	*≤0.1*
Hair Dyes and colors	1073	82	1	5	76
1989 Totals		82	1	5	76

Consumers are strongly urged to patch test for sensitivity 24 h prior to every application of the hair dye. Instructions are to apply a few drops of the hair dye with a cotton swab to a small area (the size of a quarter) behind either ear or inside the elbow. This area is to be left uncovered and undisturbed for 24 h and then evaluated for irritation.[10]

International

Diaminophenols are accepted for use by the European Economic Community,[11] providing their concentration does not exceed 10% free base. Diaminophenols can be used singly or in combination, provided that the sum of the ratios of the concentrations of each diaminophenol used in the cosmetic product expressed with reference to the maximum concentration authorized for each of them does not exceed 1.

The Japan Ministry of Health and Welfare has approved 2,4-Diamino Phenol Hydrochloride for quasidrug use only, and proper ingredient labeling is required.[12]

GENERAL BIOLOGY

Dermal Absorption

Female hairless Wistar rats were used to determine the penetration of ^{14}C-2,4-DAPE (^{14}C uniformly on the ring—specific activity: 0.8 μCi/mg).[13] The penetration of ^{14}C-2,4-DAPE was determined as pure compound and in a complete commercial formulation consisting of ^{14}C-2,4-DAPE (0.40%), ^{14}C-2,4-diaminoanisole (0.33%), *p*-phenyldiamine hyddrochloride (1.8%), resorcinol (0.05%), and *m*-aminophenol (0.1%). Both the individual compound and the formulation were dissolved in a vehicle containing nonionic and amphoteric surfactants, alcohols, glycols, oleic acid, copra diethanoamide, antioxidants and complexing agents, water, and 10% aqueous ammonia. Immediately before use, the solution was mixed with an equal volume of 20% hydrogen peroxide solution. The animals were anesthetized, and 20 mg/cm^2 of compound was applied to a 25 cm^2 area of the dorsal region. The length of time the compound remained in contact with the skin was 40 min. Following the exposure period, excess test material was removed, and a stripping process was carried out on the site of application to avoid contamination of the excrement. For 4 days, the feces and total amount of urine excreted were collected at 24-h intervals and analyzed. The animals were then killed and necropsied in order to determine the quantity of compound that had been absorbed and not excreted. In the majority of cases, the visceral organs, carcasses, skin (except for the site of application), and, in certain cases, an additional number of selected organs were examined for residual radioactivity. The quantity of compound that penetrated was 5.05 nM ± 0.79 nM (0.84 μ ± 0.13 μ) of pure compound per cm^2 of skin and 2.83 nM ± 0.49 nM (0.47 μ + 0.08 μ) of compound in the commercial formulation per cm^2 of skin. Penetration of 2,4-DAPE in the commercial formulation was 40% of the penetration of the pure compound alone. The study was then carried out applying the hair dye solutions every 30 to 40 days (duration of testing not given) to simulate human hair-dying frequency. The livers and thyroids of the treated rats were examined for accumulation of test article. At the highest doses, with the animals being killed 4 days after treatment, no radioactivity appeared in the thyroid, and only trace amounts appeared in the liver.

Female hairless Wistar rats were used to measure the absorption of ^{14}C-2,4-DAPE dissolved in a commercial vehicle at concentrations of 0.40% (23.65 nM), 0.80% (47.30 nM), and 1.20% (70.95 nM).[14] The solution was mixed with an equal volume of 20% H_2O_2 before use. Twenty mg/cm^2 were applied to a 25 cm^2 area on the back of each rat, 6 rats per group, for a period of 40 min. Urine and feces were collected for 4 days after treatment, and the animals were then killed and necropsied to determine the amount of compound absorbed and not yet excreted. The penetration per cm^2 was between 5.03 nM ± 0.79 nM (0.84 μ ± 0.13 μ) for the lowest concentration and 9.42 nM ± 0.84 nM (1.58 μ ± 0.14 μ) for the highest concentration.

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

Twenty albino Swiss mice (10 males and 10 females), approximate weight 20 g each, and 20 albino Wistar rats (10 males and 10 females), approximate weight 200 g each, were orally administered a 1 ml/100 g volume of solution containing 2,4-DAPE in the chlorhydrate form by gavage.[15] The animals were fasted for 12 h and then allowed to eat and drink normally for 2 h before dosing. Dose concentrations were calculated in geometric progression. The animals were observed for 3 h following administration and during the following week. Tearing, agitation then calm, piloerection, vasoconstriction, ptosis, discolored urine, difficult breathing, salivation, and convulsions were observed in some mice. Salivation, tearing, piloerection, loss of grip reflexes, difficult breathing, shaking, bloody snouts, vasodilation, diarrhea, discolored urine, and convulsions were observed in some rats. The oral LD_{50}, calculated using the method of Litchfield and Wilcoxon,[16] was 1760 mg/kg (confidence limits of 1595–1950 mg/kg) for male mice, 1739 mg/kg (confidence limits of 1356–2232 mg/kg) for female mice, 1745 mg/kg (confidence limits of 1539–1980 mg/kg) for male and female mice, 1191 mg/kg (confidence limits of 1075–1321 mg/kg) for male rats, 1040 mg/kg (confidence limits of 883–1225 mg/kg) for female rats, and 1113 mg/kg (confidence limits of 1037–1194 mg/kg) for male and female rats.

Six groups of 10 albino male Swiss mice, 25 to 30 g, were administered 2,4-DAPE by gavage.[17] The animals received 10 ml/kg of compound, either dissolved in water or suspended in methocel at 0.5% (pH nonmodified), at concentrations of 630, 790, 1000, 1580, 2000, or 2500 mg/kg. The mice were then observed for 14 days. The oral LD_{50} was calculated[16] and determined, as an average, to be 1160 mg/kg (95% confidence limits of 850–2100 mg/kg).

Subchronic Toxicity

Oral

Six groups of BDF_1 mice and six groups of F344 rats, 10 males and 10 females per group, were administered 2,4-DAPE at concentrations of 0, 0.01, 0.03, 0.05, 0.1, and 0.2% in tap water, *ad libitum*, for a period of 12 weeks.[18] No clinical signs were observed during treatment. The survival rate was 90% for the male mice in the 0.1 and 0.2% dose groups, 100% for all remaining mice, and 100% for all rats. Two mice that

had greatly reduced body weights died while on study. Their deaths were attributed to malnutrition due to inability to drink water. Both were necropsied, and atrophy of various organs was observed. In the surviving mice, males in the 0.1 and 0.2% dose groups had reduced body weight gains. Females displayed satisfactory growth throughout the course of the experiment. Feed consumption was reduced in these two dose groups for both male and female mice. For the rats, there was a dose-dependent decrease in mean body weight gain for all treated groups; feed consumption was reduced at the 0.2% concentration. Water intake was reduced for all treatment groups, both mice and rats, when compared to the control group values. Tissue specimens from 3 males and 3 females from each group, both mice and rats, were evaluated microscopically. Abnormalities of the kidneys were found in 2 mice, lesions of pneumonia were found in 7 mice, and pigment deposits were observed in the epithelial cells of thyroid follicles in a mouse from the 0.2% group. All male and female rats in the 0.2% dose group had pigment deposits in the epithelial cells of the thyroid follicles. There was no evidence of silver or iron in the deposits in either the mice or rats.

2,4-DAPE in a 5% Tween suspension was administered by oral intubation to 20 Sprague-Dawley rats (10 males and 10 females) for a period of 3 months.[19] The dose was 56 mg/kg/day (1/20 LD_{50}) at a volume of 10 ml/kg/day. A control group of 20 rats (10 males and 10 females) received vehicle alone. Clinical observations included a dull appearance of the pelage and light brown aureolas, pelage being soiled with urine, and a brown discoloration of the urine. Body weight gain of the treated group was slightly reduced, but the difference, as compared to the controls, was not statistically significant. At necropsy, a brown discoloration of the thyroid and of the trachea at the level of the thyroid was due to the hair dye. The results of histological and clinical examination of the treated animals were normal, with the exception of an increased serum glutamic-oxaloacetic transaminase (SGOT) activity and a slight increase in serum glutamic-pyruvic transaminase (SGPT) activity, alkaline phosphatase activity, and uric acid values. However, the SGPT, alkaline phosphatase, and uric acid values were within the normal limits. The significance of the increased SGOT value was relative in comparison to the control group, and it also did not exceed the normal limits of variation. The only mortality reported was the accidental death of 1 animal while on study.

Ocular Irritation

A volume of 0.1 ml of a 4% aqueous solution (pH 2.5) of 2,4-DAPE was instilled into the conjunctival sac of one eye of 6 albino Bouscat rabbits (3 males and 3 females) and was not rinsed after administration. The other eye was untreated and served as a control.[20] This solution was considered "practically not irritating" to the eyes of rabbits, with the ocular irritation index estimated to be 1.66/110 after 24 h, 0.33/110 after 48 h, and 0/110 after 72 h, 4 days, and 7 days.

Dermal Irritation

2,4-DAPE was applied to the shaved intact and abraded skin of 6 albino Bouscat rabbits (3 males and 3 females) as a 4% solution in distilled water (pH 8.5).[21] Each animal received 5 ml of solution. This solution was determined to be "slightly irritating" to rabbit skin with a primary irritation index of 0.08/8.

Sensitization

Ten female Hartley guinea pigs were used to determine the sensitizing potential of 2,4-DAPE following a modified Magnusson and Kligman technique.[22] Before administration of the compound, two 0.2 ml injections of 50% Freund's adjuvant were administered intradermally to the site of application. The compound was moistened with a few drops of distilled water for better adherence and applied epicutaneously to a 3 cm^2 area of deeply abraded skin. The test site was covered with an occlusive patch for 48 h. On day 7, a second epicutaneous application of 25% test article in petroleum jelly was administered, and an occlusive patch was applied for 48 h. On day 21, a challenge was performed by applying 25% 2,4-DAPE in petroleum jelly to a 5 cm^2 shaved, previously untreated area of skin. This area was covered with an occlusive patch for 24 h. Five female nonsensitized guinea pigs also were treated with a 25% application of 2,4-DAPE in petroleum jelly, and the site was covered with an occlusive patch for 24 h. This group served as the control group. The excess test substance was removed after patch removal, and sensitization readings were taken 48 and 72 h after the challenge application. Erythema was observed in 3 of the 10 animals, all of which recovered within 5 days. The authors suggested, based on the results of this study, that 2,4-DAPE could have a low sensitizing potential in humans.

Reproductive Effects/Teratogenicity

A dose range-finding study was conducted to determine the concentrations of 2,4-DAPE to be used in a teratology study. 2,4-DAPE was administered by intragastric intubation on days 1 to 10, inclusive, of pregnancy to four groups (6 rats per group) of pregnant specific pathogen-free rats.[23] The dose concentrations were 0, 125, 250, and 500 mg/kg/day, and the dose volumes were calculated on days 1, 4, and 7 to adjust for change in body weight. Control animals received vehicle, distilled water, only. Clinical signs in the 500 mg/kg dose group included severely increased postdose salivation, elevated gait, stained coats, fur loss in 1 animal, and discolored urine. Similar signs were observed in the 250 mg/kg dose group, with the exceptions that they had a later onset, were not as severe, less animals were affected, and more animals exhibited fur loss. At the 125 mg/kg dose, the only signs observed were slight or moderate postdose salivation and discolored urine. Feed consumption was reduced on days 1 to 3 of dosing in the 250 and 500 mg/kg dose groups and continued throughout dosing in the 500 mg/kg group. On days 1 to 4 of dosing, there was a weight loss in the high-dose group, retarded weight gain in the middose group, and minimal retardation of weight gain in the low-dose group. During days 4 to 11, weight gains were reduced in the 250 and 500 mg/kg dose groups, and, despite some recovery, weight gain was reduced in the postdose period when compared to controls. Animals were killed on day 15, and a necropsy was performed. Except for discolored fur on 4 animals, no compound-related changes were observed.

Based on the results of the previous preliminary study performed by Bottomley et al.,[23] dose concentrations of 0, 50, 100, and 200 mg/kg/day were chosen for a complete teratology study.[23] 2,4-DAPE was administered by gavage to four groups (20 per group) of pregnant Specific Pathogen-Free rats on days 6 to 15, inclusive, of pregnancy. The controls received vehicle, distilled water, only. Each rat received 10 ml/kg of solution, and the dose volume administered was calculated on days 6, 10, and 14 to adjust for changes in body weight. Clinical signs included increased postdose

salivation and discolored urine in all dose groups and fur loss in the later stages of dosing and in the postdose period in the 200 mg/kg dose group. Body weight gain was reduced at the 100 mg/kg dose and even more at the 200 mg/kg dose. On day 20 of pregnancy, the dams were killed, and the litters were examined. With the exception of a discoloring of or loss of fur for some animals, no dose-related changes were observed at necropsy. No statistically significant changes in litter size were observed. Marginal reductions in litter size were evident in the 100 and 200 mg/kg dose groups, but were considered to be unrelated to treatment. No statistically significant differences were observed in litter and fetal mean weight values. However, lower litter and fetal mean weight values in the 200 mg/kg dose group were considered to be treatment-related due to maternal effect and effects on skeletal development. In the 200 mg/kg dose groups, there was a significant dose-related increase in the incidence of skeletal anomalies and skeletal variants. This increase was thought to be "probably related to a general nonspecific retardation of embryo/fetal development during gestation." The incidence of major malformations and minor visceral anomalies was comparable among all groups.

Pregnant C57B1/6 mice (crossed with T stock males) were used to determine the teratogenic potential of 2,4-DAPE dissolved in corn oil.[24] There were 10 animals in the low and middose groups and 18 animals in the high-dose group. The solution was topically administered to a shaved area on the back of each mouse. The volume applied was 0.2 ml at concentrations of 15, 150, or 1500 mg/kg. Negative and positive control tests were performed. Sixteen negative control animals received dermal applications of corn oil only, and 19 positive control animals were administered benzo[a]pyrene on day 10.5 of pregnancy by intraperitoneal injection. Following fetal evaluation, "no teratogenic effect" nor "any significant difference in skeletal development" was found when comparing the dose groups to the negative controls.

MUTAGENICITY

In Vitro

Mutagenicity study results are summarized in Table 3.

Chinese hamster ovary (CHO) cells were used to evaluate the mutagenic potential of 2,4-DAPE at concentrations of 0.6 and 1.2 mg/ml.[25] The tests were performed with and without metabolic activation. A positive control using 2,4-diaminoanisole dihydrochloride (2,4-DAA) was included in the study. The results obtained did not give any evidence of increase in chromosomal aberration due to 2,4-DAPE either with or without metabolic activation.

Escherichia coli strains WP2, WP2uvrA, and WP2uvrA/recA were used to test for the genotoxic potential of 2,4-DAPE.[26] Concentrations of 30, 75, 189, 754, and 2000 μg/plate were tested both with and without metabolic activation by S9 mix. An *E. coli* reversion test was performed using strains WP2 and WP2uvrA. A modified Ames test was performed in which the soft top agar contained 0.25 μg/ml of L-tryptophan instead of the histidine/biotin mixture. No increase in revertants was found with or without metabolic activation. Strains WP2, WP2uvrA, and WP2uvrA/recA were used in a DNA damage/repair test. A modified Ames test similar to the test by Darroudi et al.[25] was performed, with the exceptions that the L-tryptophan concentration was increased to 1 μg/ml, and the test solution (0.1 ml) was pipetted into a hole (1 cm in diameter) cut into the center of each plate rather than being incorporated into the soft agar. There was no

TABLE 3. 2,4-DIAMINOPHENOXYETHANOL HCl MUTAGENICITY STUDIES

Test	*Organism*	*Strain*	*Method*	*Results*	*Reference*
Chromosomal aberration test	Chinese hamster ovary cells	—	Concentrations of 0.6 and 1.2 mg/ml tested in the presence and absence of metabolic activation	Negative	25
Escherichia coli reversion test	*E. coli*	WP2, WP2uvrA	Modified Ames test in which the soft top agar contained 0.25 μg/ml of L-tryptophan instead of the histidine/biotin mixture. Concentrations of 30, 75, 189, 754, and 2000 μg/plate were tested in the presence and absence of metabolic activation	Negative	26
DNA damage/repair test	*E. coli*	WP2, WP2uvrA, WP2uvrA/recA	Modified Ames test in which the soft top agar contained 1 μg/ml of L-tryptophan instead of the histidine/biotin mixture. 0.1 ml of test solution was placed in a hole in the center of the plate instead of being incorporated in the agar and tested in the presence and absence of metabolic activation	Negative	26
Chromosomal aberration test	Human lymphocytes	—	Concentrations of 10^{-3}, 10^{-4}, and 10^{-5} tested in medium with a fixation time of 1 h	Negative	6
Reverse mutation assay	*S. typhimurium*	TA1538, TA98	Ames test at concentrations of 0, 0.65, 1.25, 2.5, 5, 10, 20, 40, 80, and 160 μg/plate tested in the presence and absence of metabolic activation promoted by phenobarbitol + β-naphthoflavone S9 mix	Negative	27

TABLE 3. *Continued*

Test	*Organism*	*Strain*	*Method*	*Results*	*Reference*
Reverse mutation assay	*S. typhimurium*	TA1538	Ames test at concentrations of 0, 1, 10, 50, 100, 500, and 1000 μg/plate tested in the presence and absence of metabolic activation promoted by Aroclor 1254 S9 mix	Negative	27
Forward mutation assay	*Schizosaccharomyces pombe*	SP ade-60/rad10-198h$^-$	Cells treated with concentrations of 0, 10, 15, 20, 30, and 40 mM tested in the presence and absence of metabolic activation promoted by phenobarbitol + β-naphthoflavone S9 mix	Negative	27
Forward mutation assay	Chinese hamster ovary cells	V79	6-Thioguanine-resistant mutant colonies were scored using concentrations of 0, 5, and 20 mM in the presence and absence of metabolic activation promoted by phenobarbitol + β-naphthoflavone S9 mix	Negative	27
Mitotic gene conversion assay	*Saccharomyces cerevisiae*	D4, Genotype α/a; ga12/+; ade2-2/ade2-1; trp5-12/trp5-27; leu1/+	Treated during growth with concentrations of 0, 10, 20, and 40 mM in the presence and absence of metabolic activation promoted by phenobarbitol + β-naphthoflavone S9 mix	Negative	27
Unscheduled DNA synthesis test	HeLa cells	Human cells obtained from a cervical carcinoma	Cells were exposed to concentrations of 0, 0.02, 0.06, and 0.2 mM	Negative	27

Ames test	*S. typhimurium*	TA1538, TA98	Concentrations of 0, 60, 120, 300, 600, and 1200 μg/plate tested in the presence and absence of metabolic activation	Negative	28
Ames test	*S. typhimurium*	TA1537, TA1538, TA98, TA1535, TA100	Concentrations of 5, 10, 20, 50, 100, 250, 500, and 1000 μg/plate tested in the presence and absence of metabolic activation	Negative	29
Ames test	*S. typhimurium*	TA1535, TA100, TA1538, TA1537, TA98	Concentrations of 5, 10, 20, 50, 100, 250, 500, and 1000 μg/plate tested in the presence and absence of metabolic activation	Negative	30
Gene reversion test	*Saccharomyces cerevisiae*	XV185-14C	Concentrations of 1000, 2000, 3000, 4000, 5000, and 6000 μg/ml tested in the presence and absence of metabolic activation following the methods of Shahin and von Borstel[31,32]	Negative	30
Gene conversion test	*Saccharomyces cervisiae*	D4	Concentrations of 100, 250, 500, 1000, 1500, 2000, and 4000 μg/ml tested in the presence and absence of metabolic activation, except 100 μg/ml was only tested without metabolic activation and 4000 μg/ml was only tested with it	Negative	30
Ames test	*S. typhimurium*	TA1535, TA1537, TA1538, TA100, TA98	A concentration range of 5-1000 μg/plate, 3 plates/dose, was tested in the presence and absence of metabolic activation	Negative	5

TABLE 3. *Continued*

Test	*Organism*	*Strain*	*Method*	*Results*	*Reference*
Ames test	*S. typhimurium* *E. coli*	TA100 WP2uvrA (pKM101)	A concentration range of 5-100 μg/plate, 3 plates/dose, was tested in the presence and absence of metabolic activation induced by 0, 4, 10, and 30% S9 mix	Negative	33
Ames test	*S. typhimurium*	TA1538, TA97, TA98	A concentration range of 5-100 μg/plate, 3 plates/dose, was tested in the presence and absence of metabolic activation induced by 0, 4, 10, and 30% S9 mix	Positive in the presence of at least 10% S9 mix	33
Ames test	*S. typhimurium*	TA1538, TA98	Concentrations of 5-100 μg/plate were tested using 30% Aroclor 1254 S9 mix	10-fold (TA1538) and 14-fold (TA98) increases at 80 μg/ml	33
Fluctuation test	*S. typhimurium*	TA1538, TA98	20 μg of an overnight Lab-M nutrient broth No. 2 shake culture, a known amt. of 2,4-DAPE, and 3 ml of 2% S9 mix added to 12 ml of Vogel-Bonner salts mix medium. Tests also run by substituting the S9 mix with 3 ml Vogel-Bonner medium. After 18 h, 1 ml supplemented Vogel-Bonner medium was added, and it was incubated for 3 days	Positive for both strains in the presence of S9 and negative for both in the absence of S9	33

Sex-linked recessive test	*Drosophila melanogaster*	Berlin K males In(1) sc^{SIL} sc^{8R}+S, sc^{S1} sc^{8} w^{a} B females (Basc)	1–2-day-old males received 2,4-DAPE in m/30 phosphate buffer via the adult feeding method[37] for 3 days. They were then mated to 3–5-day-old females. Sex-linked recessive lethals were scored in the F_2 generation, with all suspected lethals being retested	Negative	36
Mouse dominant-lethal assay	Mouse	T-strain males C57B1/6 females	Males received dermal applications at concentration range of 15–1500 mg/kg and a volume no greater than 0.5 ml/day for 5 days and were then placed with virgin females for 7-day intervals. Fourteen days after mid-week of mating, the females were killed and their uteri examined	Negative	4
Micronucleus test	Mouse	CD-1	Mice received two 24 h apart oral doses of 2,4-DAPE in distilled water at a volume of 0.1 ml/10 g body weight and concentrations of 25, 50, and 100 μg/ml. Animals were killed 6 h after second dose, and direct bone marrow smears were made and examined to determine the presence of micronucleated cells in 2000 polychromatic erythrocytes per animal, and the ratio of normchromatic to polychromatic erythrocytes was determined	No increase in micronucleated cells. Ratio of normchromatic to polychromatic erythrocytes was significantly reduced	39

TABLE 3. *Continued*

Test	*Organism*	*Strain*	*Method*	*Results*	*Reference*
Mouse spot test for somatic mutation	Mouse	T-strain males C57B1/6 females	Animals were mated. On days 8, 9, and 10 of gestation, the females received dermal applications at concentrations ranging from 15 to 1500 mg/kg. Newborns were scored for nonwhite spots on days 12 and 24 of lactation	Negative	4
Plate microbial assay	Mouse *S. typhimurium*	CD-1 TA1535, TA1537, TA98, TA100	Animals received dermal applications at concentrations ranging from 15 to 1500 mg/kg for 3 days at a volume no greater than 0.5 ml/day. Urine was collected for a 16 h period, and the bacteria received either 0.1, 0.2, 0.3 ml, or deconjugated urine. A standard Ames test was performed on the TA1538 and TA98 strains	Negative	4
Salmonella/ microsome test	Rat *S. typhimurium*	Wistar TA1538, TA98, TA100	Male rats were either dermally administered 120 mg 2,4-DAPE in 4 ml phosphate buffer for 20 min, orally administered 10 mg/kg 2,4-DAPE in distilled water, or intraperitoneally administered 100 mg/kg in 0.9% NaCl. 100, 200, or 300 μg/plate were used	Negative	29

indication of differential damage. A positive and negative control, 0.5 μg of 2-aminoanthracene and 200 μl of phosphate buffer, respectively, were included. *Salmonella typhimurium* strain TA1535 was included in the test because the *E. coli* strain WP2uvrA does not significantly react with such a low dose of 2-aminoanthracene.

A chromosomal aberration test using human lymphocytes was completed. 2,4-DAPE was administered at concentrations of 10^{-3}, 10^{-4}, and 10^{-5} M in the medium.[6] The fixation time was 24 h, and the results obtained were negative.

Assays performed using 2,4-DAPE to determine its metabolic activity included: reverse mutation assays using *S. typhimurium* strains TA1538 and TA98, forward mutation assays using the *Schizosaccharomyces pombe* strain SP ade6-60/rad10-198h$^-$ and using the V79 cell line of Chinese hamsters, mitotic gene conversion assays using the yeast *Saccharomyces cerevisiae* strain D4, genotype α/a; gal2/+; ade2-2/ade2-1; trp5-12/trp5-27; leu1/+, and an unscheduled DNA synthesis (UDS) test using the HeLa human cell line from a cervical carcinoma.[27] Methyl methanesulfonate, ethyl methanesulfonate, cyclophosphamide, hycanthone, N-nitrosodimethylamine, and 2,4-DAA were used as positive controls. All tests were performed with and without metabolic activation provided by Aroclor-1254-treated or phenobarbital + β-naphthoflavone-treated S9 mix for the reverse mutation assays and using phenobarbital + β-naphthoflavone-treated S9 mix for all remaining assays. An Ames test was performed, with and without Aroclor-treated rat liver S9 mix, using *S. typhimurium* strain TA1538 at concentrations of 0, 1, 10, 50, 100, 500, and 1000 μg/plate. Concentrations of 0, 0.65, 1.25, 2.5, 5, 10, 20, 40, 80, and 160 μg/plate were administered to strains TA1538 and TA98 in the presence of phenobarbital + β-naphthoflavone-treated S9 mix. No positive results were observed using 2,4-DAPE. In the forward mutation assay using *S. pombe*, the cells were treated with 2,4-DAPE at concentrations of 0, 10, 15, 20, 30, and 40 mM. The forward mutation assay using the Chinese hamster V79 cell line was done by scoring 6-thioguanine-resistant mutant colonies using concentrations of 0, 5, and 20 mM. Negative results were obtained for both forward mutation assays. The *S. cerevisiae* cell suspensions were treated during growth in the same manner as *S. pombe* and were exposed to 2,4-DAPE at concentrations of 0, 10, 20, and 40 mM. The HeLa cells used in the UDS test were exposed to 2,4-DAPE at concentrations of 0, 0.02, 0.06, and 0.2 mM. Results were negative for both tests.

S. typhimurium strains TA1538 and TA98 were used to test for mutagenic activity of 2,4-DAPE both with and without metabolic activation.[28] Positive controls, used successfully, were 4-nitro-*o*-phenylenediamine (NOPD) and 2,4-DAA. 2,4-DAPE was tested at concentrations of 0, 60, 120, 300, 600, and 1200 μg/plate. Negative mutagenic results were obtained both with and without metabolic activation.

An Ames test was performed using five *S. typhimurium* strains. The results are an average of two independent experiments, each using three plates/dose.[29] The tests were run in the presence and absence of metabolic activation by S9 mix. Two positive controls, 1,2-diamino-4-nitrobenzene and 2-aminoanthracene, also were used. *S. typhimurium* strains TA1537, TA1538, TA98, TA1535, and TA100 were administered compound at concentrations of 5, 10, 20, 50, 100, 250, 500, and 1000 μg/plate. No mutagenic activity due to 2,4-DAPE was observed.

Mutagenic activity of 2,4-DAPE was determined using *S. typhimurium* strains TA1535, TA100, TA1537, TA1538, and TA98 and the yeast *S. cerevisiae* strains D4 and XV185-14C.[30] The tests were carried out with and without metabolic activation by S9

mix. An Ames test was performed using all five *S. typhimurium* strains at concentrations of 5, 10, 20, 50, 100, 250, 500, and 1000 μg/plate, three plates per dose. No mutagenic activity was observed. These results are averages of two independent experiments that used three plates each. The yeast *S. cerevisiae* strain D4 was treated with compound at concentrations of 100 (only without metabolic activation), 250, 500, 1000, 1500, 2000, and 4000 (only with metabolic activation) μg/ml. The *S. cerevisiae* strain XV185-14C was treated with 2,4-DAPE at concentrations of 1000, 2000, 3000, 4000, 5000, and 6000 μg/ml and followed the test methods of Shahin and von Borstel.[31,32] No mutagenic activity was detected in either yeast strain. Positive controls were run with all tests using 1,2-diamino-4-nitrobenzene and 2-aminoanthracene.

Salmonella typhimurium strains TA1535, TA1537, TA1538, TA100, and TA98 were used to test the mutagenic activity of 2,4-DAPE both with and without metabolic activation.[5] The bacteria were exposed to 2,4-DAPE at concentrations ranging from 5 to 1000 μg/plate. Three plates were used at each dose. A positive and negative control also were used. The control compounds were not named. 2,4-DAPE did not exhibit mutagenic activity either with or without metabolic activation.

The only positive results were obtained by Venitt et al.[33] when *S. typhimurium* strains TA1538, TA97, TA98, and TA100 and *E. coli* strain WP2uvrA (pKM101) were used to test for mutagenic potential of aqueous 2,4-DAPE. Three plates were run per dose using 0, 4, 10, and 30% Aroclor 1254-induced S9 mix. A positive control, 2,4-DAA, was used. An Ames test was performed on all strains at a concentration range of 5 to 100 μg/plate. Negative results were obtained with *S. typhimurium* TA100 and *E. coli* WP2uvrA (pKM101) at all dose concentrations and with all S9 mixes. A statistically significant increase in the number of *his*$^+$ revertants was obtained with *S. typhimurium* strains TA1538, TA97, and TA98 when in the presence of 10% S9 mix. The number of 2,4-DAPE-induced revertants was greater than 3.5 times the background concentration using 20 to 40% S9 mix. The number of induced revertants also was affected by the amount of NADP in the S9 mix. The number of *his*$^+$ revertants corresponded to the amount of NADP per plate. In the second set of tests using 30% S9 mix with strains TA1538 and TA98, 10-fold and 14-fold increases, respectively, were observed at 80 μg/plate. A commercial sample of 2,4-DAPE was tested before and after purification, and the unpure sample was much more active than the pure sample.

Fluctuation tests, using *S. typhimurium* strains TA1538 and TA98, were performed by adding 20 μg of an overnight Lab-M nutrient broth No. 2 shake culture of the appropriate organism, a known amount of 2,4-DAPE, and 3 ml of 2% S9 mix to 12 ml of Vogel-Bonner salts medium containing 1% glucose, 10 μg/ml biotin, and 1.5 μg/ml histidine.[33] The tests also were performed by substituting the S9 mix with 3 ml of Vogel-Bonner medium. After 18 h of incubation, 1 ml of Vogel-Bonner medium supplemented with 1% glucose and 10 μg/ml bromcresol purple was added. The wells were then incubated for another 3 days. In the presence of S9 mix, statistically significant dose-related increases in the number of positive wells were observed for both strains. Negative results were obtained in the absence of S9 mix.

A commentary paper submitted by a number of researchers regarding the previous study by Venitt et al.[33] states that the results obtained do not significantly alter the conclusions reached by a collaborative study,[34] since the findings from the collaborative study are a result of a battery of tests, whereas Venitt's results are based on a single test.[35] This battery of tests involved a number of reputable laboratories, and no mutagenic activity of 2,4-DAPE was observed.

In Vivo

Mutagenic activity of 2,4-DAPE was evaluated by performing a sex-linked recessive lethal test using *Drosophila melanogaster*.[36] At 25°C, 2,4-DAPE was dissolved in m/30 phosphate buffer (pH 6.8) and fed by the adult feeding method[37] to 1- to-2-day-old Berlin K males for 3 days. These males were then mated individually to 3- to-5-day-old virgin In(1) sc^{SIL} sc^{8R} + S, sc^{S1} sc^{8} w^{a} B females (Basc). The mating scheme used was one 3-day brood followed by two 2-day brood periods. At the end of each period, the treated male was transferred to a new vial and mated with more 3- to-5-day-old virgin females. Six-linked recessive lethals were scored in the F_2 generation using standard procedures,[37] and all suspected lethals were retested. No increase in mutation frequency was obtained from brood-fractionating experiments.

A micronucleus test[38] was performed using CD-1 mice.[39] Two doses of 2,4-DAPE were administered orally in sterile distilled water at concentrations of 25, 50, and 100 μg/ml per administration. The doses were 24 h apart and at a volume of 0.1 ml/10 g body weight. A negative control, vehicle, was administered orally, and a positive control, mitomycin C, was administered by intraperitoneal injection. After administration, ptosis, hypopnea, and lethargy were observed in all dose groups, and all animals excreted brown-pigmented urine. The animals were killed 6 h after administration of the second dose, and direct bone marrow smears were made.[40] These slides were examined to determine the presence of micronucleated cells in 2000 polychromatic erythrocytes per animal. The ratio of normochromatic to polychromatic erythrocytes also was determined. There was no increase in incidence of micronucleated cells in any dose group, but the ratio of normochromatic to polychromatic erythrocytes was significantly increased, indicating a toxic effect.

A mouse dominant-lethal assay was conducted after dermal administration of a suspension of 2,4-DAPE in corn oil to T-strain male mice.[4] Concentrations ranging from 15 to 1500 mg/kg, at a volume no greater than 0.5 ml/day, were applied to a shaved patch on the dorsal surface of the mouse. There may have been ingestion because the application site was not covered. Two mice per group received dermal applications of either the test compound or the control, corn oil, for 5 consecutive days. Another 2 received an intraperitoneal injection of the positive control, triethylene melamine (TEM), 2 days before mating. Two days after being dosed, each male was housed with 2 virgin C57B1/6 female mice for 7 days. These females were then replaced with 2 new virgin females. This sequence was repeated for 7 weeks. Fourteen days after the midweek of mating, the females were killed, and their were uteri examined for viable and nonviable fetuses, resorption sites, and total embryos. The dominant lethality results were negative. No significant results were observed with respect to the fertility index. After 6 weeks of mating, a fertility rate of only 30% was reported in the high dose. This was not considered compound-related because the indexes for weeks 5 and 7 were normal values. The average number of embryos per pregnant female also was not significantly different.

A mouse spot test for somatic mutation was performed using C57B1/6 female and T-strain male mice.[4] The animals were mated, and a minimum of 50 females with semen plugs were used per group. On days 8, 9, and 10 of gestation, the dose groups received dermal applications of 2,4-DAPE in corn oil at concentrations ranging from 15 to 1500 mg/kg. The negative control group received vehicle only. The application site was an uncovered shave patch on the dorsal surface of the mouse. The positive controls received a single 150 mg/kg intraperitoneal injection of benz[a]pyrene on gestation day

10. All animals were allowed to deliver, and the newborns were scored for nonwhite spots on days 12 and 24 of lactation. The high-dose group reported a coat color spot frequency of 1.9%; while, the negative control reported a frequency of 0%. The historical control frequency is between 1 and 2%, Therefore, these results were considered negative. There was no reduction in fertility, and there were no midventral white spots observed. The 2,4-DAPE solution was not systemically toxic or irritating to the skin of the dosed female.

In Vivo/In Vitro

2,4-DAPE was evaluated for genetic toxicity in a plate microbial assay developed by Durston and Ames[41] using urine collected from treated male CD-1 mice.[4] The test compound, suspended in corn oil, was applied to the skin for 3 days. Concentrations ranged from 15 to 1500 mg/kg. The volume was no greater than 0.5 ml/day. The application site was uncovered so there may have been ingestion of compound. A negative control, corn oil, and positive controls, Tris(2,3 Dibromo-propyl)PO4 and 2-acetylamino-fluorene, were used. Urine was collected for an approximately 16-h period, as it was excreted into containers that were being kept at 0 to 4°C. The collected urine was divided into three portions for testing. *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 were exposed to 0.1, 0.2, or 0.3 ml of urine or to deconjugated urine. Nonactivation tests also were performed by adding urine to the appropriate tubes and pouring it over the suface of selected agar plates. Slight increases were observed when the TA100 strain was exposed to treated urine, but these increases were not statistically significant. No positive results were obtained. A standard Ames test was performed using *S. typhimurium* strains TA1538 and TA98, and negative results were obtained.

A *Salmonella*/microsome test was performed using the urine of rats that were administered 2,4-DAPE in order to determine its mutagenicity potential.[29] *Salmonella typhimurium* strains TA1538, TA98, and TA100 were used. Male Wistar rats, 3 rats per group, were either topically, orally, or intraperitoneally administered 2,4-DAPE. Topical administration was made by applying 4 ml of phosphate buffer containing 120 mg of 2,4-DAPE to a 55.4 ± 8.7 cm^2 area of the back for 20 min. The compound was then removed by shampooing and thorough rinsing. One group of rats was given orally 10 ml of distilled water containing 100 mg/kg 2,4-DAPE, and another group received 10 ml intraperitoneal injections of 0.9% NaCl containing 100 mg/kg 2,4-DAPE. Negative controls using no urine or urine from rats given oral doses of 10 ml distilled water/kg were run. Positive controls were treated with 2,4-diaminoanisole. Urine was collected at −40°C for 24 h. The volume of urine that was used for each group was 100, 200, and 300 µl/plate. No mutagenic activity was detected.

CARCINOGENICITY

Three groups of BDF_1 mice, 50 males and 50 females per group, were used to determine the carcinogenic effect of 2,4-DAPE.[18] 2,4-DAPE was administered in tap water, *ad libitum*, at concentrations of 0, 0.04, and 0.07% for a period of 104 weeks. These doses were chosen by having first performed the subacute toxicity test on mice,[18] which is described earlier in this report. There were no significant differences observed in body weight, organ weight, or survival rate between treated and control

mice. At the termination of the study, gross and histopathological examinations were performed. There was no significant difference observed in target organs or tumor incidence when comparing the treated and control groups. Pigment deposits in epithelial cells of thyroid follicles, which were histochemically negative for silver and iron and were unrelated to tumor incidence, were observed in both treated groups. The chronic administration of 2,4-DAPE produced "no carcinogenic effect in mice."

Three groups of F344 rats, 50 males and 50 females per group, received 2,4-DAPE in tap water, *ad libitum,* at concentrations of 0, 0.05, and 0.1% for a period of 104 weeks.[18] The concentrations were determined by having first performed the subacute toxicity test on rats that was described earlier in this report. No dose was administered to the males in the 0.1% dose group during weeks 12 to 16 and to neither males nor females in that same dose group during weeks 32 to 36 due to a marked decrease in weight gain when compared to the controls. Mean body weight gains for both treated groups, males and females, were reduced when compared to the controls. There was no significant difference in survival rate between treated and control groups. After termination of treatment, necropsy and microscopic evaluation was performed on organs of all rats. There were no differences observed in the incidence or type of neoplasms between treated and control rats. Rats in the 0.1% dose group had pigment deposits in epithelial cells of thyroid follicles. These deposits were histochemically negative for silver and iron and were not related to the incidence of neoplasms. The researchers determined that, in rats, 2,4-DAPE produced no carcinogenic effect.

SUMMARY

2,4-DAPE is an aromatic amine that is an odorless white, slightly gray, or lavender gray powder. 1,3-Diaminobenzene, 2,4-diamino-1-methoxybenzene and 2,4-diamino-1-ethoxybenzene are not detected as impurities in 2,4-DAPE.

2,4-DAPE is used as a coupler mixed with primary intermediates in permanent (oxidative) hair dyes. In 1989, it was reported to the FDA as being used in 82 hair dye and color formulations at concentrations up to 5%, with the predominant concentration of use being $\leq 0.1\%$.

Coal tar hair dyes, including those containing 2,4-DAPE, are exempt from the principal adulteration provision and the color additive provisions in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The following caution statement should be displayed conspicuously on the label of coal tar hair dyes:

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

Dermal absorption of radioactive 2,4-DAPE, as a pure compound and in a formulation, was determined. Twenty mg/cm^2 of compound was applied; 5.05 nM $\pm$ 0.79 nM of pure compound/cm^2 and 2.83 nM $\pm$ 0.49 nM of compound in the complete formulation/cm^2 penetrated. In another dermal absorption study, solutions of radioactive 2,4-DAPE at concentrations of 0.40 to 1.20% were applied at a volume of 20

mg/cm^2. Penetration per cm^2 ranged from 5.03 nM ± 0.79 nM to 9.42 nM ± 0.84 nm depending on concentration.

The results of two acute toxicity studies found 2,4-DAPE to be slightly toxic in rats and mice according to the methods of Hodge and Sterner.[42] No clinical signs were observed in a subchronic toxicity test using concentrations of 2,4-DAPE ranging from 0.01 to 0.2% in tap water. In a subchronic study in which 56 mg/kg/day were administered in solution at a volume of 10 ml/kg/day, a dull appearance of the pelage and light brown aureolas, pelage being soiled with urine, and a brown discoloration of urine were observed. 2,4-DAPE was practically nonirritating when a 4% aqueous solution was instilled into the conjunctival sacs of the eyes of rabbits. 2,4-DAPE was slightly irritating to the skin of rabbits when a 4% solution was used. When evaluating the sensitizing potential of 2,4-DAPE, erythema was observed for 30% of the guinea pigs following the challenge. All of the animals recovered within 5 days. No teratogenic effects were observed due to administration of 2,4-DAPE.

In 22 mutagenicity studies, no mutagenic activity attributable to 2,4-DAPE was observed. In a few mutagenic assays, some mutagenic activity of 2,4-DAPE was observed.

In 2 oral carcinogenic studies, 1 in which tap water containing concentrations of 0.04 and 0.07% 2,4-DAPE was administered to mice for 104 weeks and the other in which tap water containing concentrations of 0.05 and 1.0% 2,4-DAPE was given to rats for 104 weeks, no carcinogenic effects were produced by 2,4-DAPE.

DISCUSSION

2,4-DAPE was slightly irritating to the skin of rabbits. In a sensitization study using guinea pigs, erythema was observed in 3 of 10 guinea pigs, all of which recovered in 5 days. Based on the results of this study, investigators suggested that 2,4-DAPE could have a low sensitizing potential in humans. The Expert Panel used these results, due to a lack of human data, in its safety assessment of 2,4-DAPE. Hair dyes containing 2,4-DAPE are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when cautionary statements and patch test instructions are conspicuously displayed on the labels. Prophetic patch testing of hair dye formulations with open patches in less predictive of skin reactions than patch testing with closed patches. False negative reactions may occur. Some persons may be sensitized, even under the proper conditions of use.

CONCLUSION

On the basis of the data presented in this report, the CIR Expert Panel concludes that 2,4-Diaminophenoxyethanol Dihydrochloride is safe as a cosmetic ingredient in the present practices of use and concentration.

ACKNOWLEDGMENT

Monice M. Zondlo, Scientific Analyst and Writer, prepared the literature review and technical analysis used in developing this report.

REFERENCES

1. COMITE DE LIAISON DES ASSOCIATIONS EUROPEENNES DE L'INDUSTRIE DE LA PARFUMERIE, DES PRODUITS COSMETIQUES ET DE TOILETTE (COLIPA). (1988). General data on the hair dye ingredient. 1-β-Hydroxyethyloxy-2,4-diaminobenzene, dihydrochloride. COLIPA: Brussels, Belgium. Unpublished data.*
2. ESTRIN, N.F., CROSLEY, P.A., and HAYNES, C.R. (Editors). (1982). *CTFA Cosmetic Ingredient Dictionary*, 3rd ed. Washington, DC: Cosmetic, Toiletry, and Fragrance Association, Inc., p. 75.
3. COSMAIR, Inc. (1989). Specifications of Imexine OAJ. Clark, NJ: Cosmair. Unpublished data.*
4. BRUSICK, D.J., JAGANNATH, D.R., and MATHESON, D. (1982). The activity of 2-(2',4'-diaminophenoxy)ethanol in three genetic toxicity bioassays. Mutat. Res **102,** 361–72.
5. SHAHIN, M.M., BUGAUT, A., and KALOPISSIS, G. (1983). Relationships between the chemical structure and mutagenic activity of monocylic aromatic amines. Chem. Mutagens **8,** 151–81.
6. KALOPISSIS, G. (1981). Experimental studies on a new hair dyeing ingredient 2-(2',4'-diaminophenoxy)ethanol: Lack of genotoxic properties. Toxicol. Eur. Res. III No 4.
7. FOOD AND DRUG ADMINISTRATION (FDA). (1989). Cosmetic product formulation data. FDA computer printout.
8. CODE OF FEDERAL REGULATIONS. (1982, revised as of April 1, 1984). Title 21, Part 720.4. Information requested about cosmetic products.
9. FEDERAL REGISTER. (Oct. 16, 1979). Cosmetic product warning statements; Coal tar hair dyes containing 4-methoxy-M-phenylenediamine (2,4-diaminoanisole) or 4-methoxy-M-phenylenediamine sulfate (2,4-diaminoanisole sulfate). **44**(201), 59509–10.
10. CLAIROL LOVING CARE. (1968–81). No peroxide color-lotion.
11. EUROPEAN ECONOMIC COMMUNITY (EEC). (1986). The EEC Cosmetics Directive. Updated Version—incorporating all amendments until 15th June 1986. Annex III/p. 3.
12. COSMETIC, TOILETRY, AND FRAGRANCE ASSOCIATION (CTFA). (1984). *CTFA List of Japanese Cosmetic Ingredients*. Washington, DC: CTFA, p. 22.
13. TSOMI, V., and KALOPISSIS, G. (1982). Penetration cutanee de quelques teintures capillaires chez le rat hairless. (Translated from French.) Toxicol. Eur. Res. IV, No 3, 119–27.
14. TSOMI, V., and KALOPISSIS, G. (1982). Penetration percutanee sur rat hairless de deux colorants pour cheveux (coupleurs): diamino-2-4-anisole et (diamino-2',4'-phenoxy)-2-ethanol. (Translated from French.) Toxicol. Eur. Res. IV, No 4, 205–8.
15. DOSSOU, K.G. (June 1, 1977). Acute oral toxicity test of 2,4-Diaminophenoxyethanol HCl (in chlorhydrate form). Aulnay-sous-Bois, France. L'OREAL, Corporate Research Management. Unpublished data.*
16. LITCHFIELD, J.T., and WILCOXON F. (1949). A simplified method of evaluating dose effect experiments. J. Pharmacol. Exp. Ther. **96,** 99–113.
17. SEGRE, P. (Oct. 3, 1976). Acute oral toxicity test of substance 7038 V (1-(2-hydroxy-ethyloxy)-2,4-diamino-benzene). Universita di Siena—Instituto di Farmacologia. Unpublished data.*
18. KUWABARA, N., KODAMA, K., TANAKA, N., OKABE, M., and OKAMOTO, Y. (April 15, 1983). Carcinogenicity test of Imexine OAJ. Tokyo, Japan: The Japanese Foundation for Cancer Research and the Kobayashi Kosei Research Laboratory, Ltd. Unpublished data.*
19. FOURNIER, P.E. (Oct. 10, 1978). Chronic toxicity study in the rat for Hairdye "D" (2,4-Diaminophenoxyethanol HCl). Asnieres/Seine, France: L'OREAL Laboratories, Societe de Recherches Biologiques. Unpublished data.*
20. DOSSOU, K.G. (Feb. 2, 1979). Determination of ocular irritation index for 2,4-Diaminophenoxyethanol HCl. Aulnay-sous-Bois Cedex, France: L'OREAL, Laboratories de Recherche Fondamentale. Unpublished data.*
21. DOSSOU, K.G. (Jan. 31, 1979). Primary cutaneous irritation of 2,4-Diaminophenoxyethanol HCl at 4% in distilled water with an 8.5 pH. Aulnay-sous-Bois, France: L'OREAL, Laboratoires de Recherche Fondamentale. Unpublished data.*
22. FOURNIER, P.E. (May 10, 1978). Dermal sensitization test in the guinea pig using the product 2,4-Diaminophenoxyethanol HCl. Paris, France: Unite de Pharmacologie Clinique Hopital Fernand Widal. Unpublished data.*
23. BOTTOMLEY, A.M., PALMER, A.K., CLARK, R., BARTON, S.J., and SIMONS, R. (1981). Effect of 2,4-Diaminophenoxyethanol, Dihydrochloride (OREX 108) on pregnancy of the rat. Cambridgeshire, England: Huntingdon Research Centre. February 5, 1981 report LRL/7/801091. Unpublished data.*
24. BELILES, R.P., et al. (1978). Teratology evaluation of OREX 108 (2,4-Diaminophenoxyethanol, Dihydrochloride) in mice. Kensington, MD: Litton Bionetics Inc. LBI Project 20807–Nov. Unpublished data.*
25. DARROUDI, F., VAN KESTEREN-VAN LEEUWEN, A.C., and NATARAJAN, A.T. (1982). Test for induction of chromosomal aberrations in Chinese hamster ovary cells (*in vitro*) by 2-(2',4'-diaminophenoxy)ethanol. Mutat. Res. **102,** 351–5.
26. HASTWELL, R.M., and McGREGOR, D.B. (1982). Lack of genotoxic activity of 2-(2',4'-diaminophenoxy)ethanol in *Escherichia coli* strains WP2, WP2uvrA, and WP2uvrA/recA. Mutat. Res. **102,** 309–12.

*Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, N.W., Suite 310, Washington, DC 20036.

27. LOPRIENO, N., BARALE, R., MARINAI, L., and ZACCARO, L. (1982). Mutagenic studies on the hair dye 2-(2',4'-diaminophenoxy)ethanol with different genetic systems. Mutat. Res. **102,** 331–46.
28. MOHN, G., BOUTER, S., and de KNIJFF, P. (1982). Mutagenic activity of 2-(2',4'-diaminophenoxy)ethanol in strains TA1538 and TA98 of *Salmonella typhimurium*. Mutat. Res. **102,** 313–8.
29. SHAHIN, M.M., ROUERS, D., BUGAUT, A., and KALOPISSIS, G. (1980). Structure–activity relationships within a series of 2,4-Diaminoalkoxybenzene compounds. Mutat. Res. **79,** 289–306.
30. SHAHIN, M.M., BUGAUT, A., and KALOPISSIS, G. (1982). Mutagenic evaluation of the hair-dye component 2,4-Diaminophenoxyethanol in *Salmonella typhimurium*/microsome plate test and *Saccharomyces cerevisiae* strains D4 and XV185-14C. Mutat. Res. **102,** 319–29.
31. SHAHIN, M.M., and VON BORSTEL, R.C. (1976). Genetic activity of the antimicrobial food additives AF-2 and H-193 in *Saccharomyces cerevisiae*. Mutat. Res. **38,** 215–24.
32. SHAHIN, M.M., and VON BORSTEL, R.C. (1977). Mutagenic and lethal effects of α-benzene hexachloride, dibutyl phthalate and trichoroethylene in *Saccharomyces cerevisiae*. Mutat. Res. **48,** 173–80.
33. VENITT, S., CROFTON-SLEIGH, C., and OSBORNE, M.R. (1983). The hair dye reagent 2-(2',4'-diaminophenoxy)ethanol is mutagenic to *Salmonella typhimurium*. Mutat. Res. **135,** 31–47.
34. COLLABORATIVE STUDY on 2-(2',4'-diaminophenoxy)ethanol. (1982). Mutat. Res. **102,** 305–72.
35. BARALE, R., BLIJLEVEN, W.G.H., BRUSICK, D.J., DARROUDI, F., KALOPISSIS, G., McGREGOR, D.B., LOPRIENO, N., MOHN, G., NATARAJAN, A.T., RICHOLD, M., SHAHIN, M.M., and SOBELS, F.H. (1983). A battery approach versus a single test for evaluating the genotoxic activity of 2-(2',4'-diaminophenoxy)ethanol. A comment on the preceding paper by Venitt, Crofton-Sleigh, and Osborne. Mutat. Res. **135,** 49.
36. BLIJLEVEN, W.G.H. (1982). Mutagenicity of 2-(2',4'-diaminophenoxy)-ethanol in *Drosophila melanogaster*. Mutat. Res. **102,** 347–9.
37. WURGLER, F.E., SOBELS, F.H., and VOGEL, E. (1977). *Drosophila* as an assay system for detecting genetic changes. In: B.J. Kilbey, M. Legator, W. Nichols, and C. Ramels (Editors). *Handbook of Mutagenicity Test Procedures*. Amsterdam: Elsevier, pp. 335–75.
38. SCHMID, W. (1975). The micronucleus test. Mutat. Res. **31,** 9.
39. RICHARDSON, J.C., and RICHOLD, M. (1982). An evaluation of the mutagenic potential of 2-(2',4'-diaminophenoxy)ethanol using the micronucleus test. Mutat. Res. **102,** 357–60.
40. HEDDLE, J.A. (1973). A rapid *in vivo* test for chromosomal damage. Mutat. Res. **18,** 187–90.
41. DURSTON, W.E., and AMES, B.N. (1974). A simple method for the detection of mutagens in urine. Studies with the carcinogen 2-acetylaminofluorene. Proc. Natl. Acad. Sci. **71,** 737–41.
42. HODGE, H.C., and STERNER, J.H. (1949). Tabulation of toxicity classes. Am. Indus. Hyg. A. Quart. **10,** 93–6.

Final Amended Report of the Cosmetic Ingredient Review Expert Panel

Amended Safety Assessment of 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate

December 12, 2007

The 2007 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Christina L. Burnett, CIR Scientific Analyst/Writer.

Cosmetic Ingredient Review
1101 17th Street, NW, Suite 412 ◊ Washington, DC 20036-4702 ◊ ph 202.331.0651 ◊ fax 202.331.0088 ◊ cirinfo@cir-safety.org

1101 17th Street, NW, Suite 412
Washington, DC 20036

Final Report of the Amended Safety Assessment of 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate

Abstract: 2,4-Diaminophenoxyethanol HCl is an aromatic amine salt that is an odorless white, slightly gray, or lavender gray powder. 2,4-Diaminophenoxyethanol Sulfate also is an aromatic amine salt for which no chemical, physical, or toxicological data were available. 2,4-Diaminophenoxyethanol HCl is soluble in water and DMSO up to 10% (w/w), but is insoluble in solvents such as acetone and propylene glycol. 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are used as couplers mixed with primary intermediates in permanent (oxidative) hair dyes. Both ingredients are used in concentrations up to 2%. These ingredients are accepted for use in the European Union and Japan. Dermal absorption of 2,4-Diaminophenoxyethanol is low. The results of 3 acute oral toxicity studies found 2,4-Diaminophenoxyethanol HCl to have an oral LD_{50} ranging from 1160 to 1760 mg/kg in mice and 1000 to 1191 mg/kg in rats. No clinical signs were observed in one subchronic toxicity test using mice and rats and only discoloration was observed in rats in another study. In another subchronic oral toxicity study using rats, a NOEL of 20 mg/kg/day was reported for 2,4-Diaminophenoxyethanol HCl. At 100 mg/kg/day, a higher incidence of hemosiderosis (without associated hematological changes), increased salivation, lower weight gain in males, colored urine, traces of nitrites and bilirubin in urine. 2,4-Diaminophenoxyethanol HCl was practically nonirritating when a 4% aqueous solution was instilled into the conjunctival sacs of the eyes of rabbits in two studies. When tested neat using rabbits, it was found to be an ocular irritant. 2,4-Diaminophenoxyethanol HCl was slightly irritating to the skin of rabbits when a 4% solution was used. When tested neat using rabbits, it also was not considered an irritant. In a guinea pig maximization study, 2,4-Diaminophenoxyethanol HCl produced erythema in 3/10 guinea pigs at challenge. In a study using a Buehler test methodology, 2,4-Diaminophenoxyethanol HCl applied neat did not produce sensitization reactions. An LLNA study of 2,4-Diaminophenoxyethanol HCl at 0.5 to 10% in DMSO did show lymphoproliferative responses indicative of delayed contact hypersensitivity. Overall, 2,4-Diaminophenoxyethanol HCl could be considered a moderate sensitizer. No teratogenic effects were observed due to administration of 2,4-Diaminophenoxyethanol HCl in an oral study using rats or in a dermal study using mice. In another rat study, an oral dose of 125 mg/kg was maternally toxic and associated with fetal weight deficits and some delayed ossification; the NOEL was 20 mg/kg. Genotoxicity assays using bacterial, mammalian cells, drosophila, mice, and rats provided mixed results. In most bacterial assays, the results were negative, but an increase in mutation frequency was reported in two studies using *S. typhimurium* TA98 with metabolic activation. In mammalian cell assays, results were negative, except for one study which found an increase in micronucleated cells in human lymphocytes that were mitogen-stimulated for 48 h (but no increase with 24 h mitogen stimulation). In animal assays (dominant lethal, micronucleus, unscheduled DNA synthesis), no evidence of genotoxicity was reported. In 2 oral carcinogenic studies, treatment of mice and rats with 2,4-Diaminophenoxyethanol HCl produced no carcinogenic effects. No human studies were available that specifically addressed these two ingredients. Available epidemiology studies that consider the possible link between hair dye use and bladder cancer, lymphoma and leukemia, other cancers, reproductive and developmental outcomes, and other endpoints were described. These data were considered insufficient to conclude there is a causal relationship between hair dye use and cancer and other endpoints, based on lack of strength of the associations and inconsistency of findings. Hair dyes containing these ingredients, as coal tar hair dye products, should have labeling which includes a caution statement and patch test instructions for determining whether the product causes skin irritation. Following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposure. While no toxicity studies were identified specifically for the sulfate salt, the toxicities of the two salts are expected to be the same, and their maximum use concentrations are the same, so exposures as used in hair dyes would be the same. Therefore, these two chemicals are safe as hair dye ingredients in the practices of use and concentration as described in this safety assessment.

INTRODUCTION

A safety assessment for 2,4-Diaminophenoxyethanol Dihydrochloride was published by the Cosmetic Ingredient Review (CIR) Expert Panel in 1991 with the conclusion that this ingredient is "safe as a cosmetic ingredient in the present practices of use and concentration" (Elder 1991). In the *International Cosmetic Ingredient Dictionary and Handbook*, 2,4-Diaminophenoxyethanol Dihydrochloride is now called 2,4-Diaminophenoxyethanol HCl (Gottschalck and McEwen 2006).

2,4-Diaminophenoxyethanol Sulfate has been added to the safety assessment. While no toxicity data were available for the sulfate salt, it is not considered likely that the toxicity of the sulfate is significantly different from that of the

hydrochloride salt or the free base. Both of these ingredients are used as couplers in permanent (oxidative) hair dyes.

CHEMISTRY

Definition and Structure

According to the *International Cosmetic Ingredient Dictionary and Handbook*, 2,4-Diaminophenoxyethanol HCl (CAS No. 66422-95-5) is the aromatic amine salt that conforms to the structure shown in Figure 1 (Gottschalck and McEwen 2006).

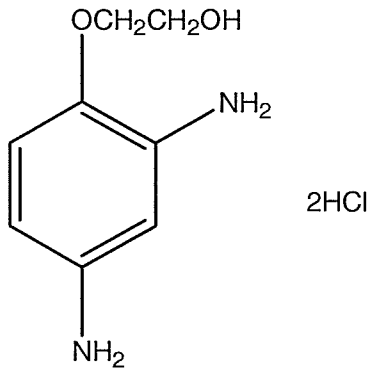


Figure 1. Chemical structure for 2,4-Diaminophenoxyethanol HCl

A technical name/synonym is Ethanol, 2-(2,4-Diaminophenoxy)-, Dihydrochloride (Gottschalck and McEwen 2006).

Trade names include:

- Colorex OAJ,
- Imexine OAJ,
- Jarocol DPE (2 HCl),
- Rodol 24 Dape, and
- Velsol Blue A42.

2,4-Diaminophenoxyethanol Sulfate (CAS No. 70643-20-8) is the substituted aromatic amine that conforms to the structure shown in Figure 2 (Gottschalck and McEwen 2006).

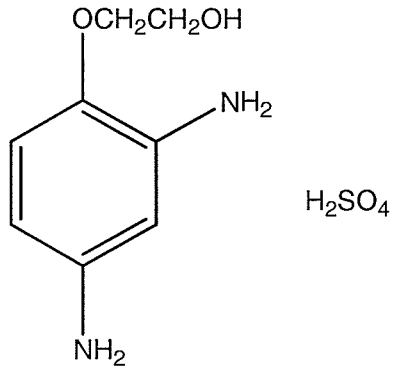


Figure 2. Chemical structure for 2,4-Diaminophenoxyethanol Sulfate

Technical names include:

- Ethanol, 2-(2,4-Diaminophenoxy)-, Sulfate (1:1) (Salt), and
- [4-(2-Hydroxyethoxy)-1,3-Phenylene]-Diammonium Phosphate.

A trade name is JAROCOL DPE (Gottschalck and McEwen 2006).

Properties

2,4-Diaminophenoxyethanol HCl is an odorless white, slightly gray powder that is soluble in water and insoluble in acetone and 95% ethanol. Physical and chemical properties of 2,4-Diaminophenoxyethanol HCl are summarized in Table 1. No physical or chemical properties were found for 2,4-Diaminophenoxyethanol Sulfate.

Analytical Methods

Groult (2004) described an analytical method for detection of 2,4-Diaminophenoxyethanol HCl using high performance liquid chromatography with UV radiation detection (300 nm). The accuracy of the method was >100 ± 10% and the imprecision was ≤10%. The author reported that this method can quantify 2,4-Diaminophenoxyethanol HCl over a concentration range of 5 - 100 μg/ml. The limit of detection was 0.005 mg/ml in purified water and 0.05 mg/ml in DMSO.

Impurities

1,3-Diaminobenzene, 2,4-diamino-1-methoxybenzene, and 2,4-diamino-1-ethoxybenzene are not detected as impurities in 2,4-Diaminophenoxyethanol HCl (Cosmair, Inc. 1989). No information regarding impurities that may be present in 2,4-Diaminophenoxyethanol Sulfate was found.

USE

Cosmetic

According to the *International Cosmetic Ingredient Dictionary and Handbook*, 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate function as hair colorants (Gottschalck and McEwen 2006), and the reported product categories are hair dyes and colors (all types requiring caution statements and patch tests).

Industry reports to the Food and Drug Administration (FDA) under the FDA's Voluntary Product Registration Program (VCRP) for 2,4-Diaminophenoxyethanol HCl in 2006 indicated 115 total cosmetic uses, all as hair dyes and colors (FDA 2006). 2,4-Diamino-phenoxyethanol Sulfate was reported to have a total of 5 uses, all in hair dyes and colors (FDA 2006).

An industry survey conducted by the Cosmetic, Toiletry, and Fragrance Association (CTFA) reported current use concentration ranges for 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate to be 0.05 - 2% and 0.4 - 2%, respectively (CTFA 2007).

Table 1. Chemical and Physical Properties of 2,4-Diaminophenoxyethanol HCl

Property	Description	Reference
Physical appearance	white powder, slightly gray	Cosmair, Inc. 1989
	lavender-gray powder	Brusick et al. 1982
Odor	None	Cosmair, Inc. 1989
Molecular weight	241	COLIPA 1988
Empirical formula	$C_8H_{12}N_2O_2 \cdot 2ClH$	Gottschalck and McEwen 2006
Melting point	198 - 216°C	Shahin et al. 1983
Free base melting point	84°C	Kalopissis 1981
Solubility	water - soluble at 25°C	Cosmair, Inc. 1989
	95% ethanol - insoluble at 25°C	
	acetone - insoluble at 25°C	
	acetone/olive oil - insoluble	Groult 2004
	dimethylformamide - insoluble	
	methyl ethyl ketone - insoluble	
	propylene glycol - insoluble	
	DMSO - 10% after 30 min stirring	
	10% in water	Toner 2005
Spectrum absorbance (λ_m - absorbancy)	238 ± 5 - 0.630 ± 0.050 286 ± 5 - 0.260 ± 0.020	Cosmair, Inc. 1989
Decomposition point (thermopan miscroscope)	198 - 217°C	COLIPA 1988
Titer, potentiometry	$\geq$ 99.5%	COLIPA 1988
Assay, % acid function	99 ± 1	Cosmair, Inc. 1989
Chloride concentration	28.0 - 31.0	Cosmair, Inc. 1989

Table 2 presents the current usage and use concentrations data for 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate as a function of product category.

According to COLIPA (1988) the amount of 2,4-Diaminophenoxyethanol HCl added to an oxidative hair dye formulation is based on a desired shade, with concentrations generally as follows: less than 0.025% in light tones, between 0.025 and 0.1% in medium tones, and between 0.1% and 4% in darker tones (which represents a smaller part of the market). 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are considered coal tar hair dyes for which regulations require caution statements and instructions regarding patch tests in order to be exempt from the principal adulteration provision and from the color additive provisions in §601 and §706 of the Federal Food, Drug, and Cosmetic Act of 1938 (FDA 1979).

Product labels shall bear a caution statement and patch test instructions for determining whether the product causes skin irritation. In order to be exempt, the following caution statement should be displayed conspicuously on the labels of coal tar hair dyes:

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

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

> The Cosmetic Ingredient Review (CIR) Expert Panel has reviewed the cosmetic industry's current coal tar hair dye product labeling, which recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 24 hours after application of the test material and prior to the use of a hair dye formulation.
>
> Since the recommendation on the industry's adopted labeling establishes a procedure for individual user safety testing, it is most important that the recommended procedure be consistent with current medical practice.

Table 2. Cosmetic product uses and concentrations for 2,4-Diaminophenoxyethanol salts.

Product Category	2006 uses (total number of products in a category; FDA 2006)	2007 use concentrations (CTFA 2007) (%)
	2,4-Diaminophenoxyethanol HCl	
Hair coloring products		
Dyes and colors	115 (1600)	0.05 - 2
Total uses/ranges for 2,4-Diaminophenoxyethanol HCl	**115**	**0.05 - 2***
	2,4-Diaminophenoxyethanol Sulfate	
Hair coloring products		
Dyes and colors	5 (1600)	0.4 - 2
Total uses/ranges for 2,4-Diaminophenoxyethanol Sulfate	**5**	**0.4 - 2***

*The maximum 4% concentration is reduced to a final on-head concentration of 2% when combined with hydrogen peroxide on application.

There is a consensus among dermatologists that screening patients for sensitization (allergic contact dermatitis) should be conducted by the procedures used by the North American Contact Dermatitis Group and the International Contact Dermatitis Group (North American Contact Dermatitis Group 1980; Eiermann et al. 1982; Adams et al. 1985). Basically, these procedures state that the test material should be applied at an acceptable concentration to the patient, covered with an appropriate occlusive patch, and evaluated for sensitization 48 and 72 hours after application. The CIR Expert Panel has cited the results of studies conducted by both the North American Contact Dermatitis Group and the International Contact Dermatitis Group in its safety evaluation reports on cosmetic ingredients (Elder 1985).

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

The industry was advised of this recommendation and asked to provide any compelling reasons why this recommendation should not be made by the Expert Panel and adopted by the cosmetic industry. No opposition to this recommendation was received. At the February 11, 1992 public meeting of the CIR Expert Panel, this policy statement was adopted.

In the European Union (2005), 2,4-Diaminophenoxyethanol and its salts have been listed in Annex III (part 2) as provisionally allowed substances with use as oxidizing coloring agents for hair dyeing. The maximum authorized concentration in finished cosmetic products is 4.0%, with the understanding that these dyes are used in combination with hydrogen peroxide, so that the maximum use concentration of the dye upon application is 2.0%.

According to the Ministry of Health, Labor, and Welfare (MHLW) of Japan, 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are not included on the list of ingredients that must not be used in cosmetic products that are marketed in Japan. However, in Japan, hair dyes are regulated as quasi-drugs and all ingredients, both active and inactive, must be specifically approved. 2,4-Diaminophenoxyethanol HCl is an approved hair dye active while the sulfate salt is not specifically approved (MHLW 2005).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, Excretion

Animal

Female hairless Wistar rats were used to determine the penetration of ^{14}C-2,4-Diamino-phenoxyethanol HCl (^{14}C uniformly on the ring-specific activity: 0.8 μCi/mg) (Tsomi and Kalopissis 1982a). The penetration of ^{14}C-2,4-Diaminophenoxyethanol HCl was determined as pure compound and in a complete commercial formulation consisting of ^{14}C-2,4-Diaminophenoxyethanol HCl (0.40%), ^{14}C-2,4-diaminoanisole (0.33%), p-phenyldiamine hydrochloride (1.8%), resorcinol (0.05%), and *m*-aminophenol (0.1%). Both the individual compound and the formulation were dissolved in a vehicle containing nonionic and amphoteric surfactants, alcohols, glycols, oleic acid, copra diethanoamide, antioxidants and complexing agents, water, and 10% aqueous ammonia. Immediately before use, the solution was mixed with an equal volume of 20% hydrogen peroxide solution. The animals were anesthetized, and 20 mg/cm^2 of compound was applied to a 25 cm^2 area of the dorsal region. The length of time the compound remained in contact with the skin was 40 min. Following the exposure period, excess test material was removed, and a stripping process was carried out on the site of application to avoid contamination of the excrement. For 4 days, the feces and total amount of urine excreted were collected at 24-h intervals

and analyzed. The animals were then killed and necropsied in order to determine the quantity of compound that had been absorbed and not excreted. In the majority of cases, the visceral organs, carcasses, skin (except for the site of application), and, in certain cases, an additional number of selected organs were examined for residual radioactivity.

The quantity of compound that penetrated was 5.05 ± 0.79 nM (0.84 ± 0.13 µg) of pure compound per cm^2 of skin and 2.83 ± 0.49 nM (0.47 ± 0.08 µg) of compound in the commercial formulation per cm^2 of skin. Penetration of 2,4-Diaminophenoxyethanol HCl in the commercial formulation was 40% of the penetration of the pure compound alone.

The study was then carried out applying the hair dye solutions every 30 to 40 days (duration of testing not given) to simulate human hair-dying frequency. The livers and thyroids of the treated rats were examined for accumulation of test article. At the highest doses, with the animals being killed 4 days after treatment, no radioactivity appeared in the thyroid, and only trace amounts appeared in the liver (Tsomi and Kalopissis 1982a).

Tsomi and Kalopissis (1982b) used female hairless Wistar rats to measure the absorption of [^{14}C]2,4-Diaminophenoxyethanol HCl dissolved in a commercial vehicle at concentrations of 0.40% (23.65 nM), 0.80% (47.30 nM), and 1.20% (70.95 nM). The solution was mixed with an equal volume of 20% H_2O_2 before use, and 20 mg/cm^2 was applied to a 25 cm^2 area on the back of each rat, 6 rats per group, for a period of 40 min. The animals were then killed and necropsied to determine the amount of compound absorbed and not yet excreted.

The penetration per cm^2 was between 5.03 ± 0.79 nM (0.84 ± 0.13 µg) for the lowest concentration and 9.42 ± 0.84 nM (1.58 ± 0.14 µg) for the highest concentration (Tsomi and Kalopissis 1982b).

Human

Toner (2005) determined the percutaneous absorption of 2,4-Diaminophenoxyethanol (dihydrochloride salt) using human dermatomed skin. Skin samples were obtained from breast and abdominal skin, transferred on ice, and frozen until used. Skin was dermatomed to a thickness of 370-400 microns and mounted in diffusion cells.

2-(2,4-diamino[ring-U-^{14}C]phenoxy)ethanol hydrochloride as the radioactive tracer added to 98.7% pure 2,4-Diaminophenoxyethanol (dihydrochloride salt) comprised the test material. Table 3 describes the composition of three formulations (oxidative, non-oxidative, and "placebo") used in this study. A 4th formulation was the developer only, H_2O_2 at 6%.

Table 3. Percent composition of formulations used in percutaneous absorption studies (Toner 2005).

Ingredient	Formulation 1 (oxidative)	Formulation 2 (non-oxidative)	Formulation 3 ("placebo")
Oleyl Alcohol	5.00	5.00	5.00
p-Phenylenediamine	1.79	-	-
Hexylene Glycol	9.00	9.00	9.00
Trideceth-2 Carboxamide MEA	10.00	10.00	10.00
Oleic Acid	3.00	3.00	3.00
Ammonium Hydroxide	4.12	4.12	4.12
Pentasodium Pentatate	0.96	0.96	0.96
PEG-2 Oleamine	7.00	7.00	7.00
Propylene Glycol	4.00	4.00	4.00
Alcohol Denat.	6.52	6.52	6.52
2,4-Diaminophenoxyethanol HCl	3.59	3.59	-
[^{14}C]2,4-Diaminophenoxyethanol HCl	0.41	0.41	-
Polyglyceryl-2 Oleyl Ether	4.00	4.00	4.00
Polyglyceryl-4 Oleyl Ether	5.46	5.46	5.46
Sodium Metabisulfite	0.46	0.46	0.46
Water	32.05	33.84	37.43
Erythorbic Acid	0.31	0.31	0.31
Sodium Diethylaminopropyl Cocoaspartamide	2.75	2.75	2.75

For the oxidative hair dye simulation, immediately prior to dosing, equal parts of formulation 1 and formulation 4 were combined. For the non-oxidative hair dye simulation, immediately prior to dosing, equal parts of formulation 2 and degassed water were combined.

Absorption was assessed by collecting the phosphate buffered saline receptor fluid (calcium and magnesium free) hourly from 0 to 24 h. At 30 minutes after sample application, the skin was washed with water, 2% sodium dodecyl sulphate (w/v in water), and water again. At 24 h, the underside of the skin was rinsed with receptor fluid. The skin was removed from its mounting, dried, and tape-stripped. Samples were analyzed using liquid scintillation counting. No use of formulation 3 ("placebo") was described. A total of 12 skin samples were tested using the oxidative hair dye simulation, but data from 4 of these tests were rejected due to low mass balance (not further explained). All 12 samples in the non-oxidative hair dye simulation were used.

The author defined the dislodgeable dose as the mass of test item removable from the application site at the 30 minute wash. Unabsorbed dose is the mass of test item in the dislodgeable dose + unexposed skin (under the flange of the diffusion cell) + stratum corneum. The absorbed dose is the mass of test item reaching the receptor fluid within the specified time. Dermal delivery is the sum of the absorbed dose + the mass of test material in the dermis/epidermis.

Table 4 presents the data for oxidative and non-oxidative hair dye simulations. The author summarized the data into a single dermal absorption value of 1.74 µg equiv./cm^2 for the oxidative test preparation and 6.55 µg equiv./cm^2 for the non-oxidative test preparation (Toner 2005).

ANIMAL TOXICOLOGY

Acute Toxicity

Dossou (1977) orally administered a 1 ml/100 g volume of solution containing 2,4-Diaminophenoxyethanol HCl in the chlorhydrate form by gavage to 20 albino Swiss mice (10 males and 10 females), approximate weight 20 g each, and 20 albino Wistar rats (10 males and 10 females), approximate weight 200 g each. The animals were fasted for 12 h and then allowed to eat and drink normally for 2 h before dosing. Dose concentrations were calculated in geometric progression. The animals were observed for 3 h following administration and during the following week.

Tearing, agitation then calm, piloerection, vasoconstriction, ptosis, discolored urine, difficult breathing, salivation, and convulsions were observed in some mice. Salivation, tearing, piloerection, loss of grip reflexes, difficult breathing, shaking, bloody snouts, vasodilation, diarrhea, discolored urine, and convulsions were observed in some rats.

The oral LD_{50} calculated was 1760 mg/kg (confidence limits of 1595-1950 mg/kg) for male mice, 1739 mg/kg (confidence limits of 1356-2232 mg/kg) for female mice, 1745 mg/kg (confidence limits of 1539-1980 mg/kg) for male and female mice, 1191 mg/kg (confidence limits of 1075-1321 mg/kg) for male rats, 1040 mg/kg (confidence limits of 883-1225 mg/kg) for female rats, and 1113 mg/kg (confidence limits of 1037-1194 mg/kg) for male and female rats (Dossou 1977).

Six groups of 10 albino male Swiss mice, 25 to 30 g, were administered 2,4-Diamino-phenoxyethanol HCl by gavage (Segre 1976). The animals received 10 ml/kg of compound, either dissolved in water or suspended in methocel at 0.5% (pH nonmodified), at doses of 630, 790, 1000, 1580, 2000, or 2500 mg/kg. The mice were then observed for 14 days. The oral LD_{50} was calculated and determined, as an average, to be 1160 mg/kg (95% confidence limits of 850-2100 mg/kg).

Manciaux (1998a) reported a study using Sprague-Dawley rats (5 of each sex), fasted overnight, and given 2,4-Diaminophenoxyethanol (as the dihydrochloride salt) at a dose of 1000 mg/kg in water by oral gavage (10 ml/kg). During the 14-day observation period, 1 male and 3 female rats were found dead on day 2. Death was preceded by hypoactivity, piloerection, lateral decubitus and tonic-clonic convulsions; all of which were observed in surviving animals. All surviving animals recovered fully by day 5. No findings on necropsy were reported in either the animals that died or those killed at 14 days.

Table 4. Summary of percutaneous absorption data (Toner 2005).

	Oxidative		Non-oxidative	
Site[a]	µg equiv./cm^2	% applied dose	µg equiv./cm^2	% applied dose
dislodgeable dose	379.97 ± 24.54	89.68 ± 4.06	369.63 ± 21.37	94.11 ± 4.54
unabsorbed dose	399.60 ± 26.01	94.31 ± 4.23	390.47 ± 13.49	99.44 ± 2.79
absorbed dose	0.11 ± 0.12	0.03 ± 0.03	2.94 ± 3.30	0.75 ± 0.84
dermal delivery	1.74 ± 1.08	0.41 ± 0.26	6.55 ± 4.72	1.68 ± 1.23

[a] dislodgeable dose is the mass of test item removable from the application site at the 30 minute wash; unabsorbed dose is the mass of test item in the dislodgeable dose + unexposed skin (under the flange of the diffusion cell) + stratum corneum; the absorbed dose is the mass of test item reaching the receptor fluid within the specified time; and the dermal delivery is the sum of the absorbed dose + the mass of test material in the dermis/epidermis.

Subchronic Toxicity

Kuwabara et al. (1983) administered 2,4-Diaminophenoxyethanol HCl at concentrations of 0, 0.01, 0.03, 0.05, 0.1, and 0.2% in tap water, *ad libitum*, to 6 groups of BDF_1 mice and 6 groups of F344 rats, 10 males and 10 females per group, for a period of 12 weeks.

No clinical signs were observed during treatment. The survival rate was 90% for the male mice in the 0.1 and 0.2% dose groups, 100% for all remaining mice and all rats. Two mice that had greatly decreased body weights died while on study. Their deaths were attributed to malnutrition due to inability to drink water. Both were necropsied, and atrophy of various organs was observed.

In the surviving mice, males in the 0.1 and 0.2% dose groups had decreased body weight gains. Females had satisfactory growth throughout the course of the experiment. Feed consumption was decreased in these 2 dose groups for both male and female mice. For the rats, there was a dose-dependent decrease in mean body weight gain for all treated groups; feed consumption was decreased at the 0.2% concentration. Water intake was decreased for all treatment groups, both mice and rats, when compared to the control group values.

Tissue specimens from 3 males and 3 females from each group, both mice and rats, were evaluated microscopically. Abnormalities of the kidneys were found in 2 mice, lesions of pneumonia were found in 7 mice, and pigment deposits were observed in the epithelial cells of thyroid follicles in a mouse from the 0.2% group. All male and female rats in the 0.2% dose group had pigment deposits in the epithelial cells of the thyroid follicles (Kuwabara et al. 1983).

Fournier (1978a) administered 2,4-Diaminophenoxyethanol HCl in a 5% Tween suspension by oral intubation to 20 Sprague-Dawley rats (10 males and 10 females) for a period of 3 months. The dose was 56 mg/kg/day (1/20 LD_{50}) at a volume of 10 ml/kg/day. A control group of 20 rats (10 males and 10 females) received vehicle alone.

Clinical observations included a dull appearance of the pelage and light brown areolas, pelage being soiled with urine, and a brown discoloration of the urine. Body weight gain of the treated group was slightly decreased, but the difference, as compared to the controls, was not statistically significant. At necropsy, a brown discoloration of the thyroid gland and of the trachea at the level of the thyroid gland was due to the hair dye.

The results of histological and clinical examination of the treated animals were normal, with the exception of an increased serum glutamic-oxaloacetic transaminase (SGOT) activity and a slight increase in serum glutamic-pyruvic transaminase (SGPT) activity, alkaline phosphatase activity, and uric acid values. However, the SGPT, SGOT, alkaline phosphatase, and uric acid values were within the normal limits for rats. The only mortality reported was the accidental death of 1 animal while on study (Fournier 1978a).

Chevalier (2005) reported a 13-week oral toxicity study using Sprague-Dawley rats. Daily administration of 2,4-Diaminophenoxyethanol (dihydrochloride salt) by oral gavage at 0, 4, 20, or 100 mg/kg/day in 5 ml water occurred over the course of the study. Each group consisted of 10 animals of each sex. An additional 6 animals of each sex were added to the control and high dose groups and held for a 4-week recovery period after treatment. An additional 6 animals were added to each group receiving the test material and were used for toxicokinetic evaluation performed on day 1 and during week 13. Mortality determinations, clinical observations (daily and weekly), functional test battery (during week 13), body weight and food intake measurement (weekly), ocular examination, blood hematology and clinical chemistry, and urinalysis were all performed. At the end of the study, animals were killed and necropsied.

Plasma levels of 2,4-Diaminophenoxyethanol in the toxicokinetic study were not detectable in the low dose group. In the 20 mg/kg/day group, plasma levels of 2,4-Diaminophenoxyethanol were detectable at 30 minutes after dosing (no shorter determination made). In the 100 mg/kg/day group, plasma levels of 2,4-Diaminophenoxyethanol were maximal at 30 minutes. The authors cited low stability of the test material in frozen plasma as a confounding factor in interpreting the toxicokinetic data.

There were no treatment-related deaths, adverse clinical signs, or changes in food intake. In the high dose group, increased salivation was observed. Body weights were decreased in the high dose group during the dosing period, but returned to normal at the end of the recovery period. Urine discoloration, traces of glucose/nitrates, and bilirubin were seen in the high dose group, but disappeared at the end of the recovery period. No increases in plasma glucose or bilirubin were seen.

Isolated statistically significant organ weight differences were reported including: 10% increase for relative brain weight in high dose males (but not females); and 10% increase for relative kidney weight in high dose females (but not males). After the recovery period, absolute (13%) and relative (15%) kidney weights were increased in females (but not males) and absolute (43%) and relative (52%) thymus weights were increased in females (but not males). No associated pathology was found in any of these organs.

Brown discoloration of thyroid glands and brown pigment seen on tissue examination in the high dose group was reported and this persisted to the end of the recovery period. No inflammatory, degenerative, or proliferative changes were seen in the thyroid gland histology.

Splenic hemosiderosis in the high dose group that persisted to the end of the recovery period was reported, but there were no associated hematological changes. The author reported the NOEL to be 20 mg/kg/day (Chevalier 2005).

Ocular Irritation

Dossou (1979a) instilled 0.1 ml of a 4% aqueous solution (pH 2.5) of 2,4-Diaminophenoxyethanol HCl into the conjunctival sac of 1 eye of 6 albino Bouscat rabbits (3 males and 3 females) and was not rinsed after administration. The other eye was untreated and served as a control. This solution was considered "practically not irritating" to the eyes of rabbits, with the ocular irritation index estimated to be 1.66/110 after 24 h, 0.33/110 after 48 h, and 0/110 after 72 h, 4 days, and 7 days.

Besson (1991a) reported a study using 3 female New Zealand White rabbits in which a 100 mg sample of 2,4-Diaminophenoxyethanol (dihydrochloride salt) was instilled neat into the conjunctival sac of the left eye (eyes were not rinsed). The right eye served as the control. Ocular effects were determined at 1, 24, 48, and 72 h post exposure and at days 8 and 15. Marked chemosis, slight to moderate conjunctival redness, slight to moderate corneal opacification, and slight iridal lesions were observed. While lessened, these effects had not disappeared after 15 days. The material as tested was considered an ocular irritant.

Sire (2004) used 3 female New Zealand White rabbits in which a 0.1ml aliquot of a 4% dilution of 2,4-Diaminophenoxyethanol (dihydrochloride salt) in water was instilled into the conjunctival sac of the left eye (eyes were not rinsed). The right eye served as the control. Ocular effects were determined at 1, 24, 48, and 72 h post exposure. Slight chemosis and redness were observed in 2/3 animals in the treated eyes, but the effect did not persist beyond day 2. There were no corneal or iridal effects. The material, tested as a 4% dilution in water, was not considered an ocular irritant.

Dermal Irritation

According to Dossou (1979b), 2,4-Diaminophenoxyethanol HCl was applied to the shaved intact and abraded skin of 6 albino Bouscat rabbits (3 males and 3 females) as a 4% solution in distilled water (pH 8.5). Each animal received 5 ml of solution. This solution was determined to be "slightly irritating" to rabbit skin with a primary irritation index of 0.08/8.

Besson (1991b) used 3 female New Zealand white rabbits in a dermal irritation study. A 0.5 g sample of 2,4-Diaminophenoxyethanol (dihydrochloride salt) was applied to a clipped area of the right flank, that had been moistened with 0.5 ml of paraffin oil. A non-occlusive dressing was applied for 4 h and then removed. At 1, 24, 48, and 72 h after the dressing was removed, cutaneous reactions were assessed on the treated and control (untreated left flank). Slight erythema was observed in 1 animal at the treatment site at the 48 h observation. No other effects were seen and the material, as tested, was not considered a dermal irritant.

Dermal Sensitization

Fournier (1978b) used 10 female Hartley guinea pigs to determine the sensitizing potential of 2,4-Diaminophenoxyethanol HCl following a modified Magnusson and Kligman technique. Before administration of the compound, two 0.2 ml injections of 50% Freund's adjuvant were administered intradermally to the site of application. The compound was moistened with a few drops of distilled water for better adherence and applied epicutaneously to a 3 cm^2 area of deeply abraded skin. The test site was covered with an occlusive patch for 48 h. On day 7, a second epicutaneous application of 25% test article in petroleum jelly was administered, and an occlusive patch was applied for 48 h.

On day 21, a challenge was performed by applying 25% 2,4-Diaminophenoxyethanol HCl in petroleum jelly to a 5 cm^2 shaved, previously untreated area of skin. This area was covered with an occlusive patch for 24 h. Five female nonsensitized guinea pigs also were treated with a 25% application of 2,4-Diaminophenoxyethanol HCl in petroleum jelly, and the site was covered with an occlusive patch for 24 h. This group served as the control group. The excess test substance was removed after patch removal, and sensitization readings were taken 48 and 72 h after the challenge application.

Erythema was observed in 3 of the 10 animals, all of which recovered within 5 days. The authors suggested, based on the results of this study, that 2,4-Diaminophenoxyethanol HCl could have a low sensitizing potential in humans (Fournier 1978b).

Manciaux (1998b) used 15 Dunkin-Hartley guinea pigs in a sensitization study. Animals were clipped and/or shaved at the anterior left flank treatment site before each application. Three induction exposures, using 10 male and 10 female guinea pigs, were done using a gauze pad moistened with 500 mg (neat) 2,4-Diaminophenoxyethanol (dihydrochloride salt). Five control animals of each sex received gauze pads with 0.5 ml water (control) on days 1, 8, and 15. The pads were held in place for 6 h using an occlusive dressing and sites were observed at 24 h after dressing removal. On day 29, control and treated animals received a topical challenge of 0.5 ml water applied to the left flank and 500 mg of the test material to the previously unexposed right flank. Sites were observed at 24, 48, and 72 h post-challenge.

The test material colored the skin purple. The author reported finding slight erythema in 2/20 (one of each sex) test animals at the 48 h time only. The authors concluded that the test material did not produce sensitization reactions (Manciaux 1998b).

Local Lymph Node Assay (LLNA)

Sire (2005) reported an LLNA in which 28 female CBA/J mice were divided into 5 treatment, 1 negative and 1 positive control groups. Treatment groups received 2,4-Diaminophenoxyethanol (dihydrochloride salt) at 0.5, 1.0, 2.5, 5, and 10% (w/v) in DMSO. The negative control received DMSO only and the positive control received α-hexylcinnamaldehyde at 25% (w/v) in DMSO. All exposures

were made to each ear at 25 µl of material per ear for 3 consecutive days. Ear thickness was measured on days 1, 2, 3, and 6. On day 6, a single injection of [^{3}H] methyl thymidine was given. Around 5 h after injection, animals were killed, the auricular lymph nodes were excised and pooled for each group, and radioactivity measured. The stimulation index (SI = treated animal/control animal radioactivity levels) and EC_3 theoretical concentration were calculated.

Treatment-related, but not dose-related ear swelling was noted. The SI for the 0.5% group was 0.92, the 1% group was 1.56, and the 2.5% group was 1.17. These were not considered positive. Positive lymphoproliferative responses were seen for the 5% group (SI = 4.21) and the 10% group (SI = 7.42). For comparison, the positive control SI was 8.51. The authors concluded that 2,4-Diaminophenoxyethanol (dihydrochloride salt) induces delayed contact hypersensitivity in the murine LLNA (Sire 2005) .

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

A dose range-finding study was conducted to determine the concentrations of 2,4-Diaminophenoxyethanol HCl to be used in a teratology study by Bottomley et al. (1981). 2,4-Diaminophenoxyethanol HCl was administered by intragastric intubation on days 1 through 10 of pregnancy to 4 groups (6 rats per group) of pregnant specific pathogen-free rats. The doses were 0, 125, 250, and 500 mg/kg/day, and the dose volumes were calculated on days 1, 4, and 7 to adjust for change in body weight. Control animals received vehicle, distilled water, only.

Clinical signs in the 500 mg/kg dose group included severely increased post-dose salivation, elevated gait, stained coats, fur loss in 1 animal, and discolored urine. Similar signs were observed in the 250 mg/kg dose group, with the exceptions that they had a later onset, were not as severe, less animals were affected, and more animals exhibited fur loss. At the 125 mg/kg dose, the only clinical signs observed were slight or moderate post-dose salivation and discolored urine.

Feed consumption was decreased on days 1 to 3 of dosing in the 250 and 500 mg/kg dose groups and continued throughout dosing in the 500 mg/kg group. On days 1 to 4 of dosing, there was a weight loss in the high-dose group, retarded weight gain in the mid-dose group, and minimal retardation of weight gain in the low-dose group. During days 4 to 11, weight gains were reduced in the 250 and 500 mg/kg dose groups, and, despite some recovery, weight gain was reduced in the post-dose period when compared to controls.

Animals were killed on day 15, and necropsied. Except for discolored fur on 4 animals, no compound-related changes were observed.

Based on the results of the preliminary study, doses of 0, 50, 100, and 200 mg/kg/day were chosen for a complete teratology study. 2,4-Diaminophenoxyethanol HCl was administered by gavage to 4 groups (20 per group) of pregnant specific pathogen-free rats on days 6 through 15 of pregnancy. The controls received vehicle, distilled water, only. Each rat received 10 ml/kg of solution, and the dose volume administered was calculated on days 6, 10, and 14 to adjust for changes in body weight.

Clinical signs included increased post-dose salivation and discolored urine in all dose groups and fur loss in the later stages of dosing and in the post-dosing period in the 200 mg/kg dose group. Body weight gain was decreased at the 100 mg/kg dose and even more at the 200 mg/kg dose.

On day 20 of pregnancy, the dams were killed, and the litters were examined. With the exception of discoloring or loss of fur for some animals, no dose-related changes were observed at necropsy.

No statistically significant changes in litter size were observed. Marginal decreases in litter size were evident in the 100 and 200 mg/kg dose groups, but were considered to be unrelated to treatment. No statistically significant differences were observed in litter and fetal mean weight values. However, lower litter and fetal mean weight values in the 200 mg/kg dose group were considered to be treatment-related due to maternal effect.

In the 200 mg/kg dose groups, there was a significant dose-related increase in the incidence of skeletal anomalies and skeletal variants. The authors stated that this increase was likely related to a nonspecific retardation of embryo/fetal development during gestation. The incidence of major malformations and minor visceral anomalies was comparable among all groups (Bottomley et al. 1981).

Pregnant C57B1/6 mice (crossed with T stock males) were used to determine the teratogenic potential of 2,4-Diaminophenoxyethanol HCl dissolved in corn oil (Beliles et al. 1978). There were 10 animals in the low and mid-dose groups and 18 animals in the high-dose group. The solution was topically administered to a shaved area on the back of each mouse. The volume applied was 0.2 ml at doses of 15, 150, or 1500 mg/kg. Negative and positive control tests were performed. Sixteen negative control animals received dermal applications of corn oil only, and 19 positive control animals were administered benzo[a]pyrene on day 10.5 of pregnancy by intraperitoneal injection. Following fetal evaluation, no teratogenic effect nor any significant difference in skeletal development, when comparing the dose groups to the negative controls, was reported.

Gaoua (2005) examined embryo/fetal developmental toxicity in female Sprague-Dawley rats exposed to 2,4-Diaminophenoxyethanol (dihydrochloride salt) daily by oral gavage from GD 6 - 19. Doses were 0, 4, 20, and 125 mg/kg/day in water (given at 5 ml/kg). Maternal clinical signs, body weight, and food intake were monitored. Dams were killed on GD 20. Gravid uterus weights were determined and fetuses were removed, sexed, weighed, and examined

externally. Implantation sites, preimplantation loss, and live and dead fetuses were recorded. Half of the fetuses were examined for soft-tissue abnormalities and half for skeletal abnormalities.

Maternal results included excessive salivation and significantly decreased body weights in the high dose group. There was no difference in the number of fetuses or in implantation sites between control and treatment groups. Only one dead fetus was reported in any group and that was in the high dose group (total fetuses 311). Fetal body weights were significantly decreased in male and female fetuses in the high dose group.

Short supernumerary 14th ribs, incomplete ossification of the centrum of the thoracic vertebrae, and incomplete ossification of the 5th sternebra were reported in the high dose group. External abnormalities were not seen in any fetus, except for one in the control group. No soft tissue malformations were seen in any group. The author reported that the NOEL for maternal and fetal developmental effects was 20 mg/kg (Gaoua 2005).

GENOTOXICITY

Genotoxicity study results are summarized in Table 5.

In Vitro Assays

Bacterial and Yeast Cell Assays

Hastwell and McGregor (1982) used *Escherichia coli* strains WP2, WP2uvrA, and WP2uvrA/recA to test for the genotoxic potential of 2,4-Diaminophenoxyethanol HCl. Concentrations of 30, 75, 189, 754, and 2000 µg/plate were tested both with and without metabolic activation by S9 mix. An *E. coli* reversion test was performed using strains WP2 and WP2uvrA.

A modified Ames test was performed in which the soft top agar contained 0.25 µg/ml of L-tryptophan instead of the histidine/biotin mixture. No increase in revertants was found with or without metabolic activation. Strains WP2, WP2uvrA, and WP2uvrA/recA were used in a DNA damage/repair test.

Another modified Ames test was performed, with the exception that the L-tryptophan concentration was increased to 1 µg/ml, and the test solution (0.1 ml) was pipetted into a hole (1 cm in diameter) cut into the center of each plate rather than being incorporated into the soft agar. A positive and negative control, 0.5 µg of 2-aminoanthracene and 200 µl of phosphate buffer, respectively, were included.

Salmonella typhimurium strain TA1535 was included in the test because the *E. coli* strain WP2uvrA did not significantly react with such a low dose of 2-amino-anthracene. There was no indication of differential damage (Hastwell and McGregor 1982).

S. typhimurium strains TA1538 and TA98 were used to test for mutagenic activity of 2,4-Diaminophenoxyethanol HCl both with and without metabolic activation (Mohn et al. 1982). Positive controls, used successfully, were 4-nitro-*o*-phenylenediamine (NOPD) and 2,4-DAA. 2,4-Diaminophenoxyethanol HCl was tested at concentrations of 0, 60, 120, 300, 600, and 1200 µg/plate. Results were negative with and without metabolic activation for 2,4-Diaminophenoxyethanol HCl.

An Ames test was performed by Shahin et al. (1980) using 5 *S. typhimurium* strains. The results are an average of 2 independent experiments, each using 3 plates/dose. The tests were run with and without metabolic activation by S9 mix. Two positive controls, 1,2-diamino-4-nitrobenzene and 2-aminoanthracene, also were used. *S. typhimurium* strains TA1537, TA1538, TA98, TA1535, and TA100 were administered compound at concentrations of 5, 10, 20, 50, 100, 250, 500, and 1000 µg/plate. No mutagenic activity due to 2,4-Diaminophenoxyethanol HCl was observed.

Shahin et al. (1982) determined mutagenic activity of 2,4-Diaminophenoxyethanol HCl using *S. typhimurium* strains TA1535, TA100, TA1537, TA1538, and TA98 and the yeast *S. cerevisiae* strains D4 and XV185-14C. The tests were carried out with and without metabolic activation by S9 mix. The test was performed using all 5 *S. typhimurium* strains at concentrations of 5, 10, 20, 50, 100, 250, 500, and 1000 µg/plate, 3 plates per dose. No mutagenic activity was observed.

S. cerevisiae strain D4 was treated with compound at concentrations of 100 (only without metabolic activation), 250, 500, 1000, 1500, 2000, and 4000 (only with metabolic activation) µg/ml. *S. cerevisiae* strain XV185-14C was treated with 2,4-Diaminophenoxyethanol HCl at concentrations of 1000, 2000, 3000, 4000, 5000, and 6000 µg/ml.

No mutagenic activity was detected in either yeast strain. Positive controls were run with all tests using 1,2-diamino-4-nitrobenzene and 2-aminoanthracene (Shahin et al. 1982).

Venitt et al.(1983) used *S. typhimurium* strains TA1538, TA97, TA98, and TA100 and *E. coli* strain WP2uvrA (pKM101) to test for mutagenic potential of aqueous 2,4-Diaminophenoxyethanol HCl. Three plates were run per dose using 0, 4, 10, and 30% Aroclor 1254-induced S9 mix. A positive control, 2,4-DAA, was used. The test was performed on all strains at a concentration range of 5 to 100 µg/plate.

Negative results were obtained with *S. typhimurium* TA100 and *E. coli* WP2uvrA (pKM101) at all dose concentrations and with all S9 mixes.

A statistically significant increase in the number of *his*$^{+}$ revertants was obtained with *S. typhimurium* strains TA1538, TA97, and TA98 when in the presence of 10% S9 mix. The number of 2,4-Diaminophenoxy-ethanol HCl-induced revertants was greater than 3.5 times the background concentration using 20 to 40% S9 mix.

The number of induced revertants also was affected by the amount of NADP in the S9 mix. The number of *his*$^+$ revertants corresponded to the amount of NADP per plate. In the second set of tests using 30% S9 mix with strains TA1538 and TA98, 10-fold and 14-fold increases, respectively, were observed at 80 µg/plate.

Fluctuation tests, using S. *typhimurium* strains TA1538 and TA98, were performed by adding 20 µg of an overnight Lab-M nutrient broth No. 2 shake culture of the appropriate organism, a known amount of 2,4-Diaminophenoxyethanol HCl, and 3 ml of 2% S9 mix to 12 ml of Vogel-Bonner salts medium containing 1% glucose, 10 µg/ml biotin, and 1.5 µg/ml histidine. The tests also were performed by substituting the S9 mix with 3 ml of Vogel-Bonner medium. After 18 h of incubation, 1 ml of Vogel-Bonner medium supplemented with 1% glucose and 10 µg/ml bromcresol purple was added. The wells were then incubated for another 3 days.

In the presence of S9 mix, statistically significant dose-related increases in the number of positive wells were observed for both strains. Negative results were obtained in the absence of S9 mix (Venitt et al. 1983).

Williams (2005) reported the results of 2,4-Diaminophenoxyethanol HCl in an Ames assay with *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102, with and without S9 metabolic activation. Using the direct plating method, concentrations of 1.6, 8, 40, 200, 1000, and 5000 µg/plate were tested and using the pre-incubation approach, concentrations of 1.3 (TA102 only), 3.3 (TA102 only), 8.2 (TA98 and TA102 only), 20.5, 51.2, 128, 320, 800, 2000, and 5000 µg/plate were tested.

The authors noted that TA98 and TA1537 are frame-shift mutations and TA100, TA1535, and TA102 are base-pair substitution mutations. Positive controls were used as follows: 2-nitrofluorene, TA98; sodium azide, TA100 and TA1535; 9-aminoacridine, TA1537; glutaraldehyde, TA102; benzo[*a*]pyrene, TA98; and 2-aminoanthracene, TA100, TA1535, TA1537, and TA102. In the plate-incorporation study, a small increase in reverse mutations was seen for strains TA98 (maximum of 90 colonies/plate) and TA102 (maximum of 292 colonies/plate) with metabolic activation.

A small increase in reverse mutations in strain TA98 (maximum of 73 colonies/plate) was seen in the pre-incubation study, in the presence of S9 metabolic activation. Positive controls yielded expected results (with S9, mean colonies/plate >400 for TA98 and >800 for TA102). The authors concluded that 2,4-Diaminophenoxyethanol HCl was a mutagen in TA98 in the presence of metabolic activation, but that the mutagenic effect was not large (Williams 2005).

Mammalian Cell Assays

Chinese hamster ovary (CHO) cells were used to evaluate the mutagenic potential of 2,4-Diaminophenoxyethanol HCl at concentrations of 0.6 and 1.2 mg/ml (Darroudi et al. 1982). The tests were performed with and without metabolic activation. A positive control using 2,4-diaminoanisole dihydrochloride (2,4-DAA) was included in the study. The authors stated that there was no evidence of increase in chromosomal aberration due to 2,4-Diaminophenoxyethanol HCl either with or without metabolic activation.

Lloyd (2005) used L5178Y mouse lymphoma cells to evaluate the mutagenic effect of 2,4-Diaminophenoxyethanol HCl with and without S9 metabolic activation in two independent experiments. In one experiment, concentrations of 400, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, and 2410 µg/ml were used in the absence of S9 and a concentration of 200 µg/ml was added to the above concentrations in the presence of S9. Positive controls were 4-nitroquinoline-1-oxide and benzo[*a*]pyrene.

In the absence of S9, cell killing precluded using the high exposure. In the presence of S9, the top two concentrations could not be used for the same reason, and the 800 µg/ml was excluded because of excessive heterogeneity. In the second experiment, concentrations of 400, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, and 2410 µg/ml were used, with or without S9.

No increase in mutations was seen at any concentration in the absence of metabolic activation in either experiment. With metabolic activation, small increases in mutation frequency was seen at 1200 and 1800 µg/ml, but not at other concentrations in the first experiment. In the second experiment, with S9 activation, small increases in mutation frequency were seen at 400, 1600, and 2410 µg/ml, but not at other concentrations.

In all cases, the small increases in mutation frequency, while higher than the controls in this study, were within the range of historical control values. This, combined with the absence of a clear dose-response, led the author to conclude that 2,4-Diaminophenoxyethanol HCl was not mutagenic in this assay, with or without metabolic activation (Lloyd 2005).

Human Lymphocytes

Kalopissis (1981) reported a chromosomal aberration test using human lymphocytes. 2,4-Diaminophenoxyethanol HCl was administered at concentrations of 10^{-3}, 10^{-4}, and 10^{-5} M in the medium. The fixation time was 24 h, and the results obtained were negative.

Kumaravel (2005) performed a chromosome aberration test in human lymphocytes in culture using 2,4-Diaminophenoxyethanol HCl. In one experiment, 987, 1234, and 1542 µg/ml of the test material was used, with and without S9 metabolic activation. In the second experiment, concentrations of 50, 77, 97, and 121 µg/ml were used in the absence of S9, and concentrations of 1408, 1760, and 2200 µg/ml were used with S9. The positive control with S9 was 4-nitroquinoline and without S9 was cyclophosphamide.

In the first experiment, pulse exposure for 3 h was used, with

and without S9. In the second experiment, a 3 h pulsed exposure was used with S9, but the cultures without S9 were exposed up until harvesting 17 h later.

Positive controls yielded expected results. Pulse-treated cells in the second experiment had frequencies of cells with structural chromosome aberrations that were significantly elevated with metabolic activation compared to controls (and were high compared to historical controls), but not without metabolic activation. Continuous exposure in the second experiment, done without metabolic activation, also yielded frequencies of cells with structural chromosome aberrations that were significantly elevated compared to controls (and were high compared to historical controls). The first experiment yielded no increases. The authors concluded that 2,4-Diaminophenoxyethanol HCl induced chromosome aberrations in cultured human lymphocytes (Kumaravel 2005).

Whitwell (2005) performed an in vitro micronucleus test in cultured human lymphocytes exposed to 2,4-Diaminophenoxyethanol HCl. Two experiments were performed, with and without S9 metabolic activation. In the first experiment, with S9, concentrations of 85, 207, 324, and 1542 µg/ml were used; and, without S9, concentrations of 106, 133, and 166 µg/ml were used. In the second experiment, with S9, concentrations of 1542, 1928, and 2410 µg/ml were used; and, without S9, concentrations of 160, 222, and 361 µg/ml were used. The positive controls, with S9, were 4-nitroquinoline and vinblastine, and, without S9, was cyclophosphamide.

Prior to incubation with the test material, cells were incubated for 24 h (experiment 1) or 48 h (experiment 2) with phytohemaglutinin. In the absence of S9, treatment was 20 h. With S9, treatment was 3 h. Cells were harvested 72 hours after initiation of treatment with test material, with the last 27 h in the presence of cytochalasin B.

Positive controls yielded expected results. No increases in micronuclei were observed in cells in experiment 1. In experiment 2, statistically significant increases in micronuclei were seen, with and without metabolic activation. Only at the highest concentrations tested were the increases outside of historical control ranges. The author concluded that, with 48h mitogen stimulation, 2,4-Diaminophenoxyethanol HCl did induce micronuclei formation in cultured human lymphocytes (Whitwell 2005).

Bacterial, Yeast, and Mammalian Cell Assays

Loprieno et al. (1982) described several assays using 2,4-Diaminophenoxyethanol HCl to determine its metabolic activity including: reverse mutation assays using *S. typhimurium* strains TA1538 and TA98; forward mutation assays using the *Schizosaccharomyces pombe* strain SP ade6-60/rad10-198h- and using the V79 cell line of Chinese hamsters; mitotic gene conversion assays using the yeast *Saccharomyces cerevisiae* strain D4, genotype α/a, gal2/+, ade2-2/ade2-1, trp5-12/trp5-27, leu1/+; and an unscheduled DNA synthesis (UDS) test using the HeLa human cell line from a cervical carcinoma.

Methyl methanesulfonate, ethyl methanesulfonate, cyclophosphamide, hycanthone, N-nitrosodimethyl-amine, and 2,4-DAA were used as positive controls. All tests were performed with and without metabolic activation provided by Aroclor-1254-treated or phenobarbital + β-naphthoflavone-treated S9 mix for the reverse mutation assays and using phenobarbital + β-naphthoflavone-treated S9 mix for all remaining assays.

The Ames test was performed, with and without Aroclor-treated rat liver S9 mix, using *S. typhimurium* strain TA1538 at concentrations of 0, 1, 10, 50, 100, 500, and 1000 µg/plate. Concentrations of 0, 0.65, 1.25, 2.5, 5, 10, 20, 40, 80, and 160 µg/plate were administered to strains TA1538 and TA98 in the presence of phenobarbital + β-naphthoflavone-treated S9 mix. No positive results were observed using 2,4-Diaminophenoxyethanol HCl.

In the forward mutation assay using *S. pombe*, the cells were treated with 2,4-Diaminophenoxyethanol HCl at concentrations of 0, 10, 15, 20, 30, and 40 mM. The forward mutation assay using the Chinese hamster V79 cell line was done by scoring 6-thioguanine-resistant mutant colonies using concentrations of 0, 5, and 20 mM. Negative results were obtained for both forward mutation assays.

The *S. cerevisiae* cell suspensions were treated during growth in the same manner as *S. pombe* and were exposed to 2,4-Diaminophenoxyethanol HCl at concentrations of 0, 10, 20, and 40 mM. The HeLa cells used in the UDS test were exposed to 2,4-Diaminophenoxyethanol HCl at concentrations of 0, 0.02, 0.06, and 0.2 mM. Results were negative for both tests (Loprieno et al. 1982).

In Vivo

Drosophila

Mutagenic activity of 2,4-Diaminophenoxyethanol HCl was evaluated by performing a sex-linked recessive lethal test using *Drosophila melanogaster* (Blijleven 1982). At 25°C, 2,4-Diaminophenoxy-ethanol HCl was dissolved in m/30 phosphate buffer (pH 6.8) and fed to 1-to-2-day-old Berlin K males for 3 days. These males were then mated individually to 3- to-5-day-old virgin In(1) sc^{SIL} sc^{8R} + S, $sc^{S1}sc^{8}$ w^{a} B females (Basc). The mating scheme used was one 3-day brood followed by two 2-day brood periods. At the end of each period, the treated male was transferred to a new vial and mated with more 3-to-5-day-old virgin females. Six-linked recessive lethals were scored in the F_2 generation and all suspected lethals were retested. No increase in mutation frequency was obtained from brood-fractionating experiments.

Mice and Rats

A micronucleus test was performed using CD-1 mice (Richardson and Richold 1982). Two doses of 2,4-Diaminophenoxyethanol HCl were administered orally in

sterile distilled water at concentrations of 25, 50, and 100 µg/ml per administration. The doses were 24 h apart and at a volume of 0.1 ml/10 g body weight. A negative control, vehicle, was administered orally, and a positive control, mitomycin C, was administered by intraperitoneal injection. After administration, ptosis, hypopnea, and lethargy were observed in all dose groups, and all animals excreted brown-pigmented urine. The animals were killed 6 h after administration of the second dose, and direct bone marrow smears were made. These slides were examined to determine the presence of micronucleated cells in 2000 polychromatic erythrocytes per animal. The ratio of normochromatic to polychromatic erythrocytes also was determined. There was no increase in the incidence of micronucleated cells in any dose group, but the ratio of normochromatic to polychromatic erythrocytes was significantly increased, indicating a toxic effect.

Brusick et al. (1982) reported a mouse dominant-lethal assay. A suspension of 2,4-Diaminophenoxyethanol HCl in corn oil was administered to the skin of T-strain male mice. Doses ranging from 15 to 1500 mg/kg, at a volume no greater than 0.5 ml/day, were applied to a shaved patch on the dorsal surface of the mouse. There may have been ingestion because the application site was not covered. Two mice per group received dermal applications of either the test compound or the control, corn oil, for 5 consecutive days. Another 2 received an intraperitoneal injection of the positive control, triethylene melamine (TEM), 2 days before mating. Two days after being dosed, each male was housed with 2 virgin C57B1/6 female mice for 7 days. These females were then replaced with 2 new virgin females. This sequence was repeated for 7 weeks. Fourteen days after the midweek of mating, the females were killed, and their uteri were examined for viable and nonviable fetuses, resorption sites, and total embryos.

The dominant lethality results were negative. No significant results were observed with respect to the fertility index. After 6 weeks of mating, a fertility rate of only 30% was reported in the high dose. This was not considered compound-related because the indexes for weeks 5 and 7 were normal values. The average number of embryos per pregnant female also was not significantly different.

These authors also performed a mouse spot test for somatic mutation using C57B1/6 female and T-strain male mice. The animals were mated, and a minimum of 50 females with semen plugs were used per group. On days 8, 9, and 10 of gestation, the dose groups received dermal applications of 2,4-Diaminophenoxyethanol HCl in corn oil at doses ranging from 15 to 1500 mg/kg. The negative control group received vehicle only. The application site was an uncovered shaved patch on the dorsal surface of the mouse. The positive controls received a single 150 mg/kg intraperitoneal injection of benz[a]pyrene on gestation day 10. All animals were allowed to deliver, and the newborns were scored for nonwhite spots on days 12 and 24 of lactation.

The high-dose group reported a coat color spot frequency of 1.9%; while the negative control reported a frequency of 0%. The author stated that the historical control frequency was between 1 and 2%, and did not consider the test material to be genotoxic in this assay. There was no reduction in fertility, and there were no midventral white spots observed. The 2,4-Diaminophenoxyethanol HCl solution was not systemically toxic or irritating to the skin of the dosed female (Brusick et al. 1982).

Erexson (2005) conducted a bone marrow micronucleus test using Sprague-Dawley rats. Rats were given 2,4-Diaminophenoxyethanol (dihydrochloride salt) by oral gavage at 0, 375, 750, or 1500 mg/kg in water (10 ml/kg). Five animals per sex were used at each dose level and an additional 6 animals per sex were added to the high dose group and 5 per sex were added to the control group. Five animals per sex were given a single dose of cyclophosphamide at 60 mg/kg as a positive control. Animals were killed 24 h after dosing, except that 5 animals per sex in the control and high dose group were retained and killed at 48 h. Bone marrow was harvested and smears prepared, stained, and scored for the number of micronucleated polychromatic erythrocytes.

The positive control yielded the expected result. Clinical signs of eye squinting were noted in the 750 mg/kg group and closed eyes, hypoactivity, and irregular breathing were noted in the high dose group. One male in the high dose group was found dead the day after dosing. The frequency of micronucleated cells in bone marrow cells was not different between control and treated animals, nor was the ratio of polychromatic cells to normochromatic cells different. The authors concluded that 2,4-Diaminophenoxyethanol (dihydrochloride salt), in rats treated up to 1500 mg/kg, did not induce micronuclei or damage bone marrow erythrocytes (Erexson 2005).

In Vivo/In Vitro

Brusick et al. (1982) evaluated excretion products of 2,4-Diaminophenoxyethanol HCl for genetic toxicity in a plate microbial assay using urine collected from treated male CD-1 mice.

The test compound, suspended in corn oil, was applied to the skin for 3 days. Doses ranged from 15 to 1500 mg/kg. The volume was no greater than 0.5 ml/day. The application site was uncovered so there may have been ingestion of compound. A negative control, corn oil, and positive controls, tris(2,3 dibromopropyl)PO4 and 2-acetylaminofluorene, were used. Urine was collected for an approximately 16-h period, as it was excreted into containers that were being kept at 0 to 4°C.

The collected urine was divided into 3 portions for testing. *S. typhimurium* strains TA1535, TA1537, TA98, and TA100 were exposed to 0.1, 0.2, or 0.3 ml of urine or to deconjugated urine. Nonactivation tests also were performed by adding urine to the appropriate tubes and pouring it over the surface of selected agar plates.

Slight increases were observed when the TA100 strain was exposed to treated urine, but these increases were not statistically significant. No positive results were obtained. A standard Ames test analyzing 2,4-Diaminophenoxyethanol HCl excretion products using *S. typhimurium* strains TA1538 and TA98 was negative (Brusick et al. 1982).

A *Salmonella*/microsome test was performed using the urine of rats that were administered 2,4-Diaminophenoxyethanol HCl in order to determine its mutagenicity potential (Shahin et al. 1980). *S. typhimurium* strains TA1538, TA98, and TA100 were used.

Male Wistar rats, 3 rats per group, were either topically, orally, or intraperitoneally administered 2,4-Diaminophenoxyethanol HCl. Topical administration was made by applying 4 ml of phosphate buffer containing 120 mg of 2,4-Diaminophenoxyethanol HCl to a 55.4 $\pm$ 8.7 cm^2 area of the back for 20 min. The compound was then removed by shampooing and thorough rinsing. One group of rats was treated orally with 10 ml of distilled water containing 100 mg/kg 2,4-Diaminophenoxyethanol HCl, and another group received 10 ml intraperitoneal injections of 0.9% NaCl containing 100 mg/kg 2,4-Diaminophenoxyethanol HCl.

Negative controls using no urine or urine from rats given oral doses of 10 ml distilled water/kg were run. Positive controls were treated with 2,4-diaminoanisole. Urine was collected at -40°C for 24 h. The volume of urine that was used for each group was 100, 200, and 300 µl/plate. No mutagenic activity was detected (Shahin et al. 1980).

Cifone (2005) conducted an in vivo/in vitro unscheduled DNA synthesis assay in Sprague-Dawley rat hepatocytes. Rats received 0, 375, 750, or 1500 mg/kg 2,4-Diaminophenoxyethanol HCl by oral gavage and were killed at 2-4 h or 14-16 h post-treatment (4 males/group/killing time). Positive controls (8) were given N-dimethylnitrosamine and killed at the early (4) or late (4) time. Hepatocytes were isolated, cultured with [^{3}H]methylthymidine for 4 h, washed, and returned to culture medium for an additional 16-20 h. Slides were prepared and autoradiographed. Net nuclear grain count and percentage of nuclei with five or more net nuclear grains were reported.

The positive control yielded the expected large increase in nuclear labeling. Clinical signs were noted at all dose levels, including hypoactivity, squinted eyes, and irregular breathing, but there were no deaths. No substantial differences were seen between hepatocytes from control and treated animals in either the mean net nuclear grain count or the mean percentage of nuclei with ≥5 grains, regardless of when the animals were killed after treatment (Cifone 2005).

Table 5. 2,4-Diaminophenoxyethanol HCl Genotoxicity Studies.

Concentration Tested	Strains Tested	Procedure	Results	Reference
Bacterial Cell Assays				
30, 75, 189, 754, and 2000 µg/plate	*E. coli* strains WP2, WP2uvrA	Modified Ames reversion test, presence and absence of metabolic activation	Negative	Hastwell & McGregor 1982
30, 75 189, 754, and 2000 µg/plate	*E. coli* strains WP2, WP2uvrA, WP2 uvrA/recA	Modified Ames reversion test, presence and absence of metabolic activation	Negative	Hastwel & McGregor 1982
0, 0.65, 1.25, 2.5, 5, 10, 20, 40, 80, and 160 µg/plate	*S. typhimurium* strains TA1538 and TA98	Ames test, presence and absence of metabolic activation	Negative	Loprieno et al. 1982
0, 1, 10, 50, 100, 500, and 1000 µg/plate	*S. typhimurium* strain TA1538	Ames test, presence and absence of metabolic activation	Negative	Loprieno et al. 1982
0, 10, 15, 20, 30, and 40 mM	*Schizosaccharomyces pombe* strain SP ade-60/rad10-198h$^-$	Forward mutation assay, presence and absence of metabolic activation	Negative	Loprieno et al. 1982
0, 10, 20, and 40 mM	*Saccharomyces cerevisiae* strains D4, genotype α/a; ga12/+; ade2-2/ade2-1; trp5-12/trp5-27; leu1/+	Mitotic gene conversion assay, presence and absence of metabolic activation	Negative	Loprieno et al. 1982

Table 5 (continued). 2,4-Diaminophenoxyethanol HCl Genotoxicity Studies.

Concentration Tested	Strains Tested	Procedure	Results	Reference
0, 60, 120, 300, 600, and 1200 µg/plate	*S. typhimurium* strains TA1538, TA98	Ames test, presence and absence of metabolic activation	Negative	Mohn et al. 1982
5, 10, 20, 50, 100, 250, 500, and 1000 µg/plate	*S. typhimurium* strains TA1537, TA1538, TA98, TA1535, TA 100	Ames test, presence and absence of metabolic activation	Negative	Shahin et al. 1980
5, 10, 20, 50, 100, 250, 500, and 1000 µg/plate	*S. typhimurium* strains TA1535, TA100, TA1538, TA1537, TA98	Ames test, presence and absence of metabolic activation	Negative	Shahin et al. 1982
1000, 2000, 3000, 4000, 5000, and 6000 µg/plate	*S. cerevisiae* strain XV185-14C	Gene reversion test, presence and absence of metabolic activation	Negative	Shahin et al. 1982
100, 250, 500, 1000, 1500, 2000, and 4000 µg/plate	*S. cerevisiae* strain D4	Gene conversion test, presence and absence of metabolic activation	Negative	Shahin et al. 1982
5 - 1000 µg/plate	*S. typhimurium* strains TA1535, TA1537, TA1538, TA100, TA98	Ames test, presence and absence of metabolic activation	Negative	Shahin et al. 1983
5 - 100 µg/plate	*S. typhimurium* strain TA100 and *E. coli* strain WP2uvrA(pKM101)	Ames test, presence and absence of metabolic activation	Negative	Venitt et al. 1983
5 - 100 µg/plate	*S. typhimurium* strain TA1538, TA97, TA98	Ames test, presence and absence of metabolic activation	Positive in the presence of at least 10% S9 mix	Venitt et al. 1983
5 - 100 µg/plate	*S. typhimurium* strains TA1538, TA98	Ames test, presence and absence of metabolic activation	10-fold (TA1538) and 14-fold (TA98) increases at 80 µg/plate with 30% S9 mix	Venitt et al. 1983
Concentration not reported	*S. typhimurium* strains TA1538, TA98	Fluctuation test, presence and absence of metabolic activation	Positive for both strains in presence of S9 and negative for both in the absence of S9	Venitt et al. 1983
1.6 - 5000 µg/plate for direct-plating; 1.3 - 5000 µg/plate for pre-incubaction	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA102	Ames test, presence and absence of metabolic activation	Positive for TA98 with S9	Williams 2005
Mammalian Cell Assays				
0.6 and 1.2 mg/ml	Chinese hamster ovary cells	Chromosomal aberration test, presence and absence of metabolic activation	Negative	Darroudi et al. 1982
10^{-3}, 10^{-4}, and 10^{-5} M	Human lymphocytes	Chromosomal aberration test	Negative	Kalopissis 1981
0, 5, and 20 mM	Chinese hamster ovary cells strain V79	Forward mutation assay, presence and absence of metabolic activation	Negative	Loprieno et al. 1982
0, 0.02, 0.06, and 0.2 mM	Human HeLa cells	Unscheduled DNA synthesis test	Negative	Loprieno et al. 1982
50 - 2200 µg/ml	human lymphocytes in culture	structural chromosome aberrations	one experiment positive, one negative; overall the author concluded the response was positive	Kumaravel 2005

Table 5 (continued). 2,4-Diaminophenoxyethanol HCl Genotoxicity Studies.

Concentration Tested	Strains Tested	Procedure	Results	Reference
200 - 2410 µg/ml	L5178Y mouse lymphoma cells	6-thioguanine resistance mutation frequency, with and without metabolic activation	isolated positive findings - not dose-dependent and within historic control values; not considered mutagenic	Lloyd 2005
85 - 2410 µg/ml	human lymphocytes in culture	micronucleated cells	with sufficient mitogen stimulation (48 h), 2,4-Diaminophenoxyethanol HCl did induce micronuclei formation in cultured human lymphocytes.; no effect with 24 h mitogen stimulation	Whitwell 2005
Drosophila				
Concentration not reported, oral	*Drosophila melanogaster* Berlin K males and ln(1)$sc^{SIL}sc^{8R}$ + S, $sc^{S1}sc^{8}w^{a}B$ females (Basc)	Sex-linked recessive test	Negative	Blijleven 1982
Animal Assays				
15 - 1500 mg/kg, dermal	Mouse, T-strain males and C57B1/6 females	Mouse dominant-lethal assay	Negative	Brusick et al. 1982
25, 50, and 100 µg/ml, oral	Mouse strain CD-1	Micronucleus test	No increase in micronucleated cells. Ratio of normochromatic to polychromatic erythrocytes was significantly reduced	Richardson & Richold 1982
15 - 1500 mg/kg, dermal	Mouse, T-strain males and C57B1/6 females	Moue spot test for somatic mutation	Negative	Brusick et al. 1982
375 - 1500 mg/kg	Sprague-Dawley rats	Unscheduled DNA synthesis	Negative	Cifone 2005
375 - 1500 mg/kg	Sprague-Dawley rats	Micronucleus test	No increase in micronucleated cells, no difference in ratio of normochromatic to polychromatic erythrocytes	Erexson 2005
Animal and Bacterial Assays				
15 - 1500 mg/kg, dermal	Mouse strain CD-1 and *S. typhimurium* strains TA1535 TA1537, TA98, TA100	Plate microbial assay	Negative	Brusick et al. 1982
100 mg/kg, dermal, oral, and intraperitoneal injection	Wistar rats and *S. typhimurium* strains TA1538, TA98, TA100	*Salmonella*/microsome test	Negative	Shahin et a. 1980

CARCINOGENICITY

Kuwabara et al. (1983) used 3 groups of BDF_1 mice, 50 males and 50 females per group, to determine the carcinogenic effect of 2,4-Diaminophenoxyethanol HCl. 2,4-Diaminophenoxyethanol HCl was administered in tap water, *ad libitum*, at concentrations of 0, 0.04, and 6.07% for a period of 104 weeks. These doses were chosen by having first performed the subacute toxicity test on mice described earlier in this report.

There were no significant differences observed in body weight, organ weight, or survival rate between treated and control mice. At the termination of the study, gross and histopathological examinations were performed.

No significant difference was observed in target organs or

tumor incidence when comparing the treated and control groups. Pigment deposits in epithelial cells of thyroid follicles, which were histochemically negative for silver and iron and unrelated to tumor incidence, were observed in both treated groups. The authors concluded that chronic administration of 2,4-Diamino-phenoxyethanol HCl produced no carcinogenic effect in mice.

These authors also reported a study in which 3 groups of F344 rats, 50 males and 50 females per group, received 2,4-Diaminophenoxyethanol HCl in tap water, *ad libitum*, at concentrations of 0, 0.05, and 0.1% for a period of 104 weeks. The concentrations were determined by having first performed the subacute toxicity test on rats that was described earlier in this report. No dose was administered to the males in the 0.1% dose group during weeks 12 to 16 and to neither males nor females in that same dose group during weeks 32 to 36 due to a marked decrease in weight gain when compared to the controls.

Mean body weight gains for both treated groups, males and females, were reduced when compared to the controls. There was no significant difference in survival rate between treated and control groups.

After termination of treatment, necropsy and microscopic evaluation was performed on organs of all rats. There were no differences observed in the incidence or type of neoplasms between treated and control rats. Rats in the 0.1% dose group had pigment deposits in epithelial cells of thyroid follicles. These deposits were histochemically negative for silver and iron and were not related to the incidence of neoplasms. The authors concluded that chronic administration of 2,4-Diaminophenoxyethanol HCl produced no carcinogenic effect in rats (Kuwabara et al. 1983).

CLINICAL ASSESSMENT OF SAFETY

No human studies were available that specifically addressed these two ingredients.

HAIR DYE EPIDEMIOLOGY

Hair dyes may be broadly grouped into oxidative (permanent) and direct (semipermanent) hair dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes are a preformed color. 2,4-Diamino-phenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate function as couplers in oxidative (permanent) hair dyes.

While the safety of individual hair dye ingredients are not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information and have been considered by the CIR Expert Panel.

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

Rollison et al. (2006) reviewed the available epidemiology literature published from1992 through February 2005, which includes over 80 citations on personal hair dye use published since the IARC review. The authors found that hair dye exposure assessment ranged from ever/never use to information on type, color, duration and frequency of use. The authors found insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. The review highlighted well-designed studies with an exposure assessment that included hair dye type, color, and frequency or duration of use, which found associations between personal hair dye use and development of acute leukemia, bladder cancer, multiple myeloma, and non-Hodgkin's lymphoma. These findings, however, were not consistently observed across studies. Several studies published since this review are described below.

Bladder Cancer - A study by Kelsey et al. (2005) was a follow-up to the previously published case-control study in New Hampshire (Andrew et al. 2004) and examined the links between those bladder cancer cases with an inactivated tumor suppressor gene (TP53) and various exposures. Huncharek and Kupelnick (2005) performed a meta-analysis of 6 case-control and 1 cohort study. Takkouche et al. (2005) performed a meta-analysis of 9 personal use case-control studies and 1 cohort study. Ji et al. (2005) reported a cohort occupational study that included hairdressers not included in the above meta-analyses. Kogevinas et al. (2006) presented evidence from a case-control study in Spain. Lin et al. (2006) presented a case-control study of personal permanent hair dye use. Serretta et al. (2006) reported preliminary results from a multicentric study of risk factors in Ta-T1 transitional cell carcinoma of the bladder, including hair dye use. Pelucchi et al. (2006) reviewed data on bladder cancer mortality rates and the recognized or potential environmental (including hair dye exposures) and genetic risk factors. Bolt and Golka (2007) reviewed the published literature on bladder cancer risk and personal use of hair dyes (17 publications) or occupation as a hairdresser and/or barber (23 publications).

Lymphoma and Leukemia - Takkouche et al. (2005) reported a meta-analysis of reports of hematopoietic cancers (19 publications). Mester et al. (2005) reviewed ten epidemiology studies regarding the relationship between occupational exposure in hairdressing and diseases of the malignant lymphoma group. A case-control study in Spain by Benavente et al. (2005) examined the association between lifetime hair dye exposure with various lymphomas, including chronic lymphocytic leukaemia. de Sanjosé et al. (2006) reported on the association between personal use of hair dyes and lymphoid neoplasm using data from a European multicenter case-control study. Chiu et al. (2007) evaluated non-Hodgkin's lymphoma subtypes defined according to the presence or absence of t(14:18) translocation as a function of smoking, familial hematopoietic cancer, and hair dye use,. Morton et al. (2007) examined the risk of non-Hodgkin's lymphoma as a function of hair dye use and genetic variation in N-acetyltransferase 1 (*NAT1*) and N-acetyltransferase 2 (*NAT2*).

Other Cancers - Takkouche et al. (2005) included breast cancer and childhood cancers in their meta-analysis. Efird et al. (2005) studied the association between the use of hair-coloring agents the month before or during pregnancy with childhood brain tumors in 1218 cases between 1976 and 1994. Heineman et al. (2005) studied 112 women in Nebraska newly diagnosed with brain cancer (glioma). McCall et al. (2005) reported on the relationship between childhood neuroblastomas and maternal hair dye use in 538 children born between 1992 and 1994 in the U.S. and Canada. Bluhm et al. (2006) reported on personal hair dye use and risks of glioma, meningioma, and acoustic neuroma. Chen et al. (2006) reported a case-control study of childhood germ cell tumors and exposure to residential chemicals, including prenatal and postnatal maternal hair dye use.

Reproductive and Developmental Outcomes - Axmon et al. (2006) compared fertility parameters in a cohort of Swedish hairdressers with matched controls. Hougaard et al. (2006) examined the risk of infertility among hairdressers in a 5-year follow-up of female hairdressers in Denmark. Zhu et al. (2006) reported on pregnancy outcomes among female hairdressers in Denmark. Thulstrup and Bonde (2006) conducted an in-depth review of 26 human studies of neural tube defects, cleft lip and cleft palate, congenital heart defects, urinary tract defects, and limb defects in which work and exposure status was known.

Other Endpoints - Park et al. (2005) reported an occupational case-control study of neurodegenerative diseases, including Alzheimer's disease, presenile dementia and motor neuron disease. Cooper et al. (2006) determined antinuclear antibody titer in individuals in the general population as a function of occupational history and ever/never use of hair dyes. Hueber-Becker et al. (2007) reported exposures of hairdressers to oxidative hair dyes (p-Phenylenediamine Hydrochloride) under controlled conditions, including estimates of systemic exposure. The authors discussed the adequacy of current safety precautions for handling hair dyes by hairdressers and the risk to health posed by the exposures found.

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

SUMMARY

2,4-Diaminophenoxyethanol HCl is an aromatic amine that is an odorless white, slightly gray, or lavender gray powder. 2,4-Diaminophenoxyethanol Sulfate also is an aromatic amine salt. No chemical, physical, or toxicological data are available on the sulfate salt. 2,4-Diaminophenoxyethanol HCl is soluble in water and DMSO up to 10% (w/w), but is insoluble in solvents such as acetone and propylene glycol.

1,3-Diaminobenzene, 2,4-diamino-1-methoxybenzene and 2,4-diamino-1- ethoxybenzene are not detected as impurities in 2,4-Diaminophenoxyethanol HCl.

2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are used as couplers mixed with primary intermediates in permanent (oxidative) hair dyes. Both ingredients are used in concentrations up to 2%.

These ingredients, as coal tar hair dye products, 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, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation.

In the European Union, 2,4-Diaminophenoxyethanol and its salts are listed in Annex III (part 2) with qualifications. In Japan, hair dyes are regulated as quasi drugs and all ingredients, both active and inactive, must be specifically approved: 2,4-Diaminophenoxyethanol HCl is an approved

hair dye active.

Dermal absorption of radioactive 2,4-Diaminophenoxyethanol HCl, as a pure compound and in an oxidative hair dye formulation, was determined using rats to be 5.05 ± 0.79 nM/cm^2 of pure compound and 2.83 ± 0.49 nM/cm^2 from the oxidative hair dye formulation (20 mg/cm^2 of compound was applied). In another dermal absorption study using rats, penetration ranged from 5.03 ± 0.79 nM/cm^2 (23.65 nM applied) to 9.42 ± 0.84 nM/cm^2 (70.95 nM applied). In an in vitro study using dermatomed human skin, the total dermal delivery from an oxidative hair dye preparation was 1.74 ± 1.08 µg equiv./cm^2, and 6.55 ± 4.72 µg equiv./cm^2 from a non-oxidative hair dye preparation.

The results of 3 acute toxicity studies found 2,4-Diaminophenoxyethanol HCl to have an LD_{50} ranging from 1160 to 1760 mg/kg in mice and 1000 to 1191 mg/kg in rats.

No clinical signs were observed in one subchronic toxicity test using mice and rats given concentrations of 2,4-Diaminophenoxyethanol HCl ranging from 0.01 to 0.2% in tap water. In a subchronic study in which 56 mg/kg/day were administered in solution to rats at a volume of 10 ml/kg/day, a dull appearance of the pelage and light brown areolas, pelage being soiled with urine, and a brown discoloration of urine were observed. In another rat study, a NOEL of 20 mg/kg/day was reported for 2,4-Diaminophenoxyethanol HCl; with brownish pigment reported in thyroid glands and a higher incidence of hemosiderosis (without associated hematological changes), increased salivation, lower weight gain in males, colored urine, traces of nitrites and bilirubin in urine at 100 mg/kg/day.

2,4-Diaminophenoxyethanol HCl was practically nonirritating when a 4% aqueous solution was instilled into the conjunctival sacs of the eyes of rabbits in two studies. When tested neat using rabbits, it was found to be an ocular irritant.

2,4-Diaminophenoxyethanol HCl was slightly irritating to the skin of rabbits when a 4% solution was used. When tested neat using rabbits, it also was not considered an irritant.

When evaluating the sensitizing potential of 2,4-Diaminophenoxyethanol HCl using a Magnusson/Kligman study design, erythema was observed in 3/10 guinea pigs at challenge with a 25% solution of test material in petrolatum. In a study using a Buehler test methodology, 2,4-Diaminophenoxyethanol HCl applied neat did not produce sensitization reactions. An LLNA study of 2,4-Diaminophenoxyethanol HCl at 0.5 to 10% in DMSO did show lymphoproliferative responses indicative of delayed contact hypersensitivity and the ingredient was considered a moderate skin sensitizer.

No teratogenic effects were observed due to administration of 2,4-Diaminophenoxyethanol HCl in an oral study using rats or in a dermal study using mice. In another rat study, an oral dose of 125 mg/kg was maternally toxic and associated with fetal weight deficits and some delayed ossification; the NOEL was 20 mg/kg.

Genotoxicity assays using bacterial, mammalian cells, drosophila, mice, and rats provided mixed results. In most bacterial assays, the results were negative, but an increase in mutation frequency was reported in two studies using *S. typhimurium* TA98 with metabolic activation. In mammalian cell assays, results were negative, except for one study which found an increase in micronucleated cells in human lymphocytes that were mitogen-stimulated for 48 h (but no increase with 24 h mitogen stimulation). In animal assays (dominant lethal, micronucleus, unscheduled DNA synthesis) no evidence of genotoxicity was reported.

The majority of the genotoxicity studies for 2,4-Diaminophenoxyethanol HCl did not observe any genotoxicity.

In 2 oral carcinogenic studies, mice and rats received 0.004 and 0.007% 2,4-Diaminophenoxy-ethanol HCl and 0.05 and 1.0% 2,4-Diaminophenoxyethanol HCl, respectively, in tap water for 104 weeks. No carcinogenic effects were reported.

Available epidemiology studies that consider the possible link between hair dye use and bladder cancer, lymphoma and leukemia, other cancers, reproductive and developmental outcomes, and other endpoints were described.

DISCUSSION

The CIR Expert Panel considered that the available acute and subchronic, oral, ocular, and dermal toxicity data are adequate to support the safety of 2,4-Diaminophenoxyethanol HCl with respect to systemic toxicity endpoints. This ingredient did not produce significant toxicity to the reproductive system or affect development of fetuses in animal studies at levels that were not maternally toxic. Based on the low dermal absorption of 2,4-Diaminophenoxyethanol HCl from oxidative hair dye formulations, such maternally toxic levels are highly unlikely. The Expert Panel noted that there were mixed results in the available genotoxicity data; however, 2,4-Diaminophenoxyethanol was not carcinogenic in mouse and rat studies.

2,4-Diaminophenoxyethanol HCl was slightly to non-irritating to the skin of rabbits. Dermal sensitization study results are mixed, but a maximization study and a local lymph node assay were positive, so this ingredient could be considered a moderate skin sensitizer. It is relevant that hair dyes containing these ingredients, as coal tar hair dye products, 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, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Expert Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposure.

No toxicity studies were identified specifically for the sulfate

salt, 2,4-Diaminophenoxyethanol Sulfate, in the published literature. The toxicities of the two salts are expected to be the same, and their maximum use concentrations are the same, so exposures as used in hair dyes would be the same. Therefore, the Expert Panel determined that the toxicity data on 2,4-Diaminophenoxyethanol HCl could be extrapolated to 2,4-Diaminophenoxyethanol Sulfate.

While there were no human studies that specifically addressed these two ingredients, the CIR Expert Panel did review the available human epidemiology data. In considering these data, the CIR Expert Panel concluded that the available hair dye epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other endpoints, based on lack of strength of the associations and inconsistency of findings. Use of direct hair dyes, while not the focus in all investigations, appears to have little evidence of any association with adverse events as reported in epidemiology studies.

CONCLUSION

On the basis of the data presented in this report, the CIR Expert Panel concludes that 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate are safe as hair dye ingredients in the practices of use and concentration as described in this safety assessment.

REFERENCES

Adams, R.M., H.I. Maibach, W.W. Clendenning, et al. 1985. A five-year study of cosmetic reactions. *J. Am. Acad. Dermatol.* 13:1062-1069.

Andrew, A.S., A.R. Schned, J.A. Heaney, and M.R. Karagas. 2004. Bladder cancer risk and personal hair dye use. *Int. J. Cancer* 109:581-586.

Axmon, A., L. Rylander, L. Lillienberg, M. Albin, and L. Hagmar. 2006. Fertility among female hairdressers. *Scand. J. Work Environ. Health.* 32(1):51-60.

Barale, R., W.G.H. Blijleven, D.J. Brusick, et al. 1983. A battery approach versus a single test for evaluating the genotoxic activity of 2-(2',4'-diaminophenoxy)ethanol. A comment on the preceding paper by Venitt, Crofton-Sleigh, and Osborne. *Mutat. Res.* 135:49.

Beliles, R.P. et al. 1978. Teratology evaluation of OREX 108 (2,4-Diaminophenoxyethanol, Dihydrochloride) in mice. Kensington, MD: Litton Bionetics Inc. LBI Project 20807-Nov. Unpublished data submitted by CTFA.[1]

Benavente, Y., N. Garcia, E. Domingo-Domenech, et al. 2005. Regular use of hair dyes and risk of lymphoma in Spain. *Int. J. Epidemiol.* 34:1118 - 1122.

Besson, C. 1991a. Imexine OAJ batch op 118: Ocular Irritation Index. CERB Study No. 910100 E. Unpublished data submitted by CTFA, August 13, 2007. 23 pages. [1]

Besson, C. 1991b. Imexine OAJ batch op 118: Primary Cutaneous Irritation. CERB Study No. 910113 E. Unpublished data submitted by CTFA, August 13, 2007. 23 pages[1].

Blijleven, W.C.H. 1982. Mutagenicity of 2-(2',4'-diaminophenoxy)-ethanol in *Drosophila melanogaster*. *Mutat. Res.* 102:347-349.

Bluhm, E.C., S.H. Zahm, H.A. Fine, et al. 2006. Personal hair dye use and risks of glioma, meningioma, and acoustic neuroma among adults. *Am. J. Epidemiol.* 165:63-71.

Bolt, H.M., and K. Golka. 2007. The debate on carcinogenicity of permanent hair dyes: New insights. *Crit. Rev. Toxicol.* 37:521-536.

Bottomley, A.M., A.K. Palmer, R. Clark, S.J. Barton, and R. Simons. 1981. Effect of 2,4-Diaminophenoxyethanol, Dihydrochloride (OREX 108) on pregnancy of the rat. Cambridgeshire, England: Huntingdon Research Centre. February 5, 1981 report LRL/7/801091. Unpublished data.[1]

Brusick, D.J., D.R. Jagannath, and D. Matheson. 1982. The activity of 2-(2',4'-diaminophenoxy)ethanol in three genetic toxicity bioassays. *Mutat. Res.* 102:361-372.

Chen, Z., L. Robison, R. Giller, M. Krailo, M. Davis, S. Davies, and X-O. Shu. 2006. Environmental exposure to residential pesticides, chemicals, dusts, funes, and metals, and risk of childhood germ cell tumors. *Int. J. Hyg. Environ. Health.* 209:31-40.

Chevalier, G. 2005. 2,4-diaminophenoxyethanol HCl (A042): 13-Week Toxicity Study by oral Route (Gavage) in Rats followed by a 4-Week Treatment-free Period. CIT Study No. 26892 TCR. Unpublished data submitted by CTFA, August 13, 2007. 20 pages. [1]

Chiu, B.C-H., B.J. Dave, A. Blair, et al. 2007. Cigarette smoking, familial hematopoietic cancer, hair dye use, and risk of t(14;18)-defined subtypes of non-Hodgkin's lymphoma. *Am. J. Epidemiol.* 165:652-659.

Cifone, M.A. 2005. 2,4-diaminophenoxyethanol HCl (A042): In vivo/in vitro Unscheduled DNA Synthesis Rat primary Hepatocyte Cultures at two Timepoints with a Dose Rangefinding Assay. Covance Study No. 6182-111.Unpublished data submitted by CTFA, August 13, 2007. 62 pages. [1].

Comite de Liaison des Associations Europeennes de l'Industrie de la Parfumerie, des Produits Cosmetiques et de Toilette (COLIPA). 1988. General data on the hair dye ingredient 1-β-Hydroxyethyloxy-2.4-diaminobenzene, dihydrochloride. COLIPA: Brussels, Belgium. Unpublished data submitted by CTFA.[1]

Cooper, G.S., C.G. Parks, P.S. Schur, and P.A. Fraser. 2006. Occupational and environmental associations with antinuclear antibodies in a general population sample. *J. Toxicol. Environ. Health. Part A.* 69:2063-2069.

Cosmair, Inc. 1989. Specifications of Imexine OAJ. Cosmair: Clark, NJ. Unpublished data submitted by CTFA.[1]

Cosmetic, Toiletry, and Fragrance Association (CTFA). 2007. Current use concentration - 2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate. Unpublished data provided by CTFA. 1 page. [1]

[1]Available for review: Director, Cosmetic Ingredient Review, 1101 17th St., NW, Suite 412, Washington DC 20036-4702.

Darroudi, F., A.C. Van Kesteren-Van Leewen, and A.T. Natarajan. 1982. Test for induction of chromosomal aberrations in Chinese hamster ovary cells (in vitro) by 2-(2',4'-diaminophenoxy)ethanol. *Mutat. Res.* 102:351-355.

de Sanjosé, S., Y. Benevente, A. Nieters, et al. 2006. Association between personal use of hair dyes and lymphoid neoplasms in Europe. *Am. J. Epidemiol.* 164:47-55.

Dossou, K.G. 1977. Acute oral toxicity test of 2,4-Diaminophenoxyethanol HCI (in chlorhydrate form). Aulnay-sous-Bois, France. L'OREAL, Corporate Research Management. Unpublished data submitted by CTFA.[1]

Dossou, K.G. 1979a. Determination of ocular irritation index for 2,4-Diaminophenoxyethanol HCl. Aulnay-sous-Bois Cedex, France: L'OREAL. Laboratoires de Recherche Fondamentale. Unpublished data submitted by CTFA.[1]

Dossou, K. G. 1979b. Primary cutaneous irritation of 2,4-Diaminophenoxyethanol HCI at 4% in distilled water with an 8.5 pH. Aulnay-sous-Bois, France: L'OREAL. Laboratoires de Recherche Fondamentale. Unpublished data submitted by CTFA.[1]

Efird, J.T., E.A. Holly, S. Cordier, et al. 2005. Beauty product-related exposures and childhood brain tumors in seven countries: Results from the SEARCH International Brain Tumor Study. *J. Neuro-Oncol.* 72:133-147.

Eiermann, H.J. W. Larsen, H.I. Maibach, et al. 1982. Prospective study of cosmetic reactions: 1977-1980. *J. Am. Acad. Dermatol.* 6:909-917.

Elder, R.L., ed.1985. Final Report on the Safety Assessment of p-Phenylenediamine. *J. Am. Coll. Toxicol.* 4(3):203-266.

Elder, R.L., ed. 1991. Final Report on the Safety Assessment of 2,4-Diaminophenoxyethanol Dihydrochloride. *J. Am. Coll. Toxicol.* 10(1):113-34.

Erexson, G.L. 2005. In vivo Rat Micronucleus Assay in 2,4-diaminophenoxyethanol HCl (A042). Covance Study No. 6182-112. Unpublished data submitted by CTFA, August 13, 2007. 53 pages. [1]

European Union. 2005. €1976, Council Directive 1976/768/EEC of 27 July 1976 on the Approximation of the Laws of the Member States Relating to Cosmetic Products, as amended through Commission Directive 2003/83/EC. Accessed October 12, 2006. http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/consleg/1976/L/01976L0768-20050913-en.pdf.

Food and Drug Administration (FDA). 1979. Cosmetic product warning statements: Coal tar hair dyes containing 4-methoxy-m-phenylenediamine (2,4-diaminanisole) or 4-methoxy-m-phenylenediamine sulfate (2,4-diaminoanisole sulfate). *Federal Register* 44:59509-59510.

FDA. 2006. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.

Fournier, P.E. 1978a. Chronic toxicity study in the rat for hair dye "D" (2,4-Diaminophenoxyethanol HCl). Asnieres/Seine. France: L'OREAL Laboratories, Societe de Recherches Biologiques. Unpublished data submitted by CTFA.[1]

Fournier, P.E. 1978b. Dermal sensitization test in the guinea pig using the product 2,4-Diaminophenoxyethanol HCl. Paris, France: Unite de Pharmacologie Clinique Hopital Fernand Widal. Unpublished data submitted by CTFA.[1]

Gaoua, W. 2005. 2,4-diaminophenoxyethanol HCl (A042): prenatal developmental Toxicity Study by oral Route (Gavage) in Rats. CIT Study No. 26891 RSR. Unpublished data submitted by CTFA, August 13, 2007. 240 pages. [1]

Gottschalck, T.E. and G.N. McEwen, Jr., eds. 2006. *International Cosmetic Ingredient Dictionary and Handbook.* 11th edn. Vol. 1. Washington, DC: CTFA. p. 655.

Groult, R. 2004. Validation of Analytical Method and Determination of Homogeneity and Stability of Dosage Forms. CIT Study No. 26963 AHS. Unpublished data submitted by CTFA, August 13, 2007. 51 pages[1].

Hastwell, R.M., and D.B. McGregor. 1982. Lack of genotoxic activity of 2-(2',4'-diaminophenoxy)ethanol in *Escherichia coli* strains WP2, WP2uvrA, and WP2uvrA/recA. *Mutat. Res.* 102:309-312.

Heineman, E.F., M.H. Ward, R. D. McComb, D.D. Weisenburger and S.H. Zahm. 2005. Hair dyes and risk of glioma among Nebraska women. *Cancer Causes Control* 16:857-864.

Hougaard, K.S., H. Hannerz, J.P. Bonde, H. Feveile, and H. Burr. 2006. The risk of infertility among hairdressers. Five-year follow-up of female hairdressers in a Danish national registry. *Human Reproduction* 21:3122-3126.

Hueber-Becker, F., G.J. Nohynek, E.K. Dufour, W.J.A. Meuling, A.Th.H.J. de Bie, H. Toutain, and H.M. Bolt. 2007. Occupational exposure of hairdressers to [^{14}C]-*para*-phenylenediamine-containing oxidative hair dyes: A mass balance study. *Food Chem. Toxicol.* 45:160-169.

Huncharek, M. and B. Kupelnick. 2005. Personal use of hair dyes and the risk of bladder cancer: Results of a meta-analysis. *Public Health Reports* 120:31-38.

International Agency for Research on Cancer (IARC). 1993. IARC Monographs on the evaluation of carcinogenic risks to humans. Vol 57. Occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines. Lyon, France: IARC. (pp. 43-118).

Ji, J., C. Granström, and K. Hemminki. 2005. Occupation and bladder cancer: A cohort study in Sweden. *Brit. J. Cancer* 92:1276-1278.

Kalopissis, C. 1981. Experimental studies on a new hair dyeing ingredient 2-(2',4'-diaminophenoxy) ethanol: Lack of genotoxic properties. *Toxicol. Eur. Res.* III No 4.

Kelsey, K.T., T. Hirao, S. Hirao, et al. 2005. TP53 alterations and patterns of carcinogen exposure in a US population-based study of bladder cancer. *Int. J. Cancer* 117(3):370-375.

Kogevinas, M., F. Fernandez, N. Garcia-Closas, et al. 2006. Hair dye use is not associated with risk for bladder cancer: Evidence from a case-control study in Spain. *Eur. J. Cancer* 42:1448-1454.

Kumaravel, T.S. 2005. 2,4-diaminophenoxyethanol HCl (A042): Induction of Chromosome Aberrations in cultured human peripheral Blood Lymphocytes. Covance Study No. 413/83. Unpublished data submitted by CTFA, August 13, 2007. 48 pages. [1]

Kuwabara, N., K. Kodama, N. Tanaka, M. Okabe, and Y. Okamoto. April 15, 1983. Carcinogenicity test of lmexine OAJ Tokyo, Japan: The Japanese Foundation for Cancer Research and the Kobayashi Kosei Research Laboratory, Ltd. Unpublished data submitted by CTFA.[1]

Lin, J., C.P. Dinney, H. B. Grossman, and X. Wu. 2006. Personal permanent hair dye use is not associated with bladder cancer risk: Evidence from a case-control study. *Cancer Epidemiol. Biomarkers Prev.* 15:1746-1749.

Lloyd, M. 2005. 2,4-diaminophenoxyethanol HCl (A042): Mutation at the hprt locus of L5178Y Mouse Lymphoma Cells using the Microtitre® Fluctuation Technique. Covance Study No. 413/85. Unpublished data submitted by CTFA, August 13, 2007. 50 pages. [1]

Loprieno, N., R. Barale, L. Marinai, and L. Zaccaro. 1982. Mutagenic studies on the hair dye 2-(2',4'-diaminophenoxy)ethanol with different genetic systems. *Mutat. Res* .102:331-346.

Manciaux, X. 1998a. Acute Oral Toxicity in Rats. CIT Study No. 17303 TAR. Unpublished data submitted by CTFA, August 13, 2007. 22 pages[1].

Manciaux, X. 1998b. Skin Sensitization Test in Guinea-pigs (Buehler Test: 3 applications). CIT Study No. 17304 TSG. Unpublished data submitted by CTFA, August 13, 2007. 32 pages. [1]

McCall, E.E., A.F. Olshan and J.L. Daniels. 2005. Maternal hair dye use and risk of neuroblastoma in offspring. *Cancer Causes Control* 16:743-8.

Mester, B., G. Elsner and A. Nienhaus. 2005. Hair dyes and malignant lymphoma - An overview of previous publications on epidemiology. *Zbl Arbeitsmed.* 55:117-125. Translated from the original German.

Ministry of Health, Labor and Welfare (MHLW). 2000. MHW Ordinance No. 332, Ingredients of quasi-drugs. Products to be used directly on the body. Ministry of Health, Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 2-2, 1-chome, Kasumigaseki, Chiyoda-ku, Tokyo 100-8045, Japan.

MHLW. 2005. MHW Ordinance No. 331, Appendix 1. List of ingredients that cosmetics shall not contain. Ministry of Health, Labor and Welfare, Pharmaceutical and Medical Safety Bureau, Inspection and Guidance Division, 2-2, 1-chome, Kasumigaseki, Chiyoda-ku, Tokyo 100-8045, Japan.

Mohn, G., S. Bouter, and P. de Knijff. 1982. Mutagenic activity of 2-(2',4'-diaminophenoxy)ethanol in strains TA1538 and TA98 of *Salmonella typhimurium. Mutat. Res.* 102:313-318.

Morton, L.M., L. Bernstein, S.S. Wang, et al. 2007. Hair dye use, genetic variation in N-acetyltransferase 1 (*NAT1*) and 2 (*NAT2*), and risk of non-Hodgkin lymphoma. *Carcinogenesis.* http://carcin.oxfordjournals.org/ Advance access published May 23, 2007.

North American Contact Dermatitis Group. 1980. Patch testing in allergic contact dermatitis. Evaston IL:American Academy of Dermatology.

Park, R.M., P.A. Schulte, J.D. Bowman, et al. 2005. Potenial occupational risks for neurodegenerative diseases. *Amer. J. Industrial Med.* 48:63-77.

Pelucchi, C., C. Bosetti, E. Negri, M. Malvezzi, and C. LaVecchia. 2006. Mechanisms of Disease: the epidemiology of bladder cancer. *Nature Clin. Practice Urol.* 3(6):1-14.

Richardson, J.C., and M. Richold. 1982. An evaluation of the mutagenic potential of 2.(2',4'-diaminophenoxy)ethanol using the micronucleus test. *Mutat. Res.* 102:357-360.

Rollison, D.E., K.J. Helzlsouer, and S.M. Pinney. 2006. Personal hair dye use and cancer: A systemic literature review and evaluation of exposure assessment in studies published since 1992. *J. Toxicol. Environ. Health. Part B* 9:413-439.

Segre, P. Oct. 3, 1976. Acute oral toxicity test of substance 7038 V (1 -(2-hydroxy-ethyloxy)-2,4-diamino-benzene). Universita di Siena-Instituto di Farmacologia. Unpublished data submitted by CTFA.[1]

Serretta, V., G. Morgia, V. Altieri, et al. 2006. Preliminary report of a multicentric study on environmental risk factors in Ta-T1 transitional cell carcinoma of the bladder. *Urol. Int.* 77:152-158.

Shahin, M.M., and R.C. Von Borstel. 1976. Genetic activity of the antimicrobial food additives AF-2 and H-193 in *Saccharomyces cerevisiae. Mutat. Res.* 38:215-224.

Shahin, M.M., and R.C. Von Borstel. 1977. Mutagenic and lethal effects of α-benzene hexachloride, dibutyl phthalate and trichloroethylene in *Saccharomyces cerevisiae. Mutat. Res.* 48:173-180.

Shahin, M.M, D. Rouers, A. Bugaut, and G. Kalopissis. 1980. Structure-activity relationships within a series of 2,4-Diaminoalkoxybenzene compounds. *Mutat. Res.* 79:289-306.

Shahin, M.M., A. Bugaut, and G. Kalopissis. 1982. Mutagenic evaluation of the hair-dye component 2,4-Diaminophenoxyethanol in *Salmonella typhimurium*: microsome plate test and *Saccharomyces cerevisiae* strains D4 and XV185-14C. *Mutat. Res.* 102:319-329.

Sire, G. 2004. 2,4-diaminophenoxyethanol HCl (A042) at the concentration of 4%; Acute Eye Irritation in Rabbits. CIT Study No. 27068 TAL. Unpublished data submitted by CTFA, August 13, 2007. 20 pages. [1]

Sire, G. 2005. 2,4-diaminophenoxyethanol HCl (A042); Evaluation of Skin Sensitization Potential in Mice using the Local Lymph Node Assay (LLNA). CIT Study No. 26964 TSS. Unpublished data submitted by CTFA, August 13, 2007. 30 pages. [1]

Takkouche, B. M., Teminan and A. Montes-Martínez. 2005. Personal use of hair dyes and risk of cancer. *JAMA.* 293(20):2516-2525.

Thulstrup, A.M. and J.P. Bonde. 2006. Maternal occupational exposure and risk of specific birth defects. *Occup. Med.* 56:532-543.

Toner, F. 2005. The In Vitro Percutaneous Absorption of Radiolabelled 2,4-Diaminophenoxyethanol HCl Through Human Skin. Inveresk Study No. 774955. Unpublished data submitted by CTFA, August 13, 2007. 86 pages[1].

Tsomi, V., and G. Kalopissis. 1982a. Penetration cutanee de quelques teintures capillaires chez le rat hairless. (Translated from French.) *Toxicol. Eur. Res.* IV, No 3. 119-127.

Tsomi, V., and G. Kalopissis. 1982b. Penetration percutanee sur rat hairless de deux colorants pour cheveux (coupleurs): diamino-2-4-anisole et (diamino-2',4'-phenoxy)-2-ethanol. (Translated from French.) *Toxicol. Eur. Res.* IV. No 4, 205-208.

Venitt, S., C. Crofton-Sleigh, and M.R. Osborne. 1983. The hair dye reagent 2-(2',4'-diaminophenoxy)ethanol is mutagenic to *Salmonella typhimurium. Mutat. Res.* 135:31-47.

Whitwell, J. 2005. 2,4-Diaminophenoxyethanol HCl (A042): Induction of Micronuclei in cultured human peripheral Blood Lymphocytes. Covance Study No. 413/84. Unpublished data submitted by CTFA, August 13, 2007. 53 pages. [1]

Williams, L. 2005. 2,4-Diaminophenoxyethanol HCl (A042): Reverse Mutation Assay in five Histidine-requiring strains of Salmonella typhimurium. Unpublished data submitted by CTFA, August 13, 2007. 58 pages. [1]

Zhu, J.L., M. Vestergaard, N.H. Hjollund, and J. Olsen. 2006. Pregnancy outcomes among female hairdressers who participated in the Danish National Birth Cohort. *Scand. J. Work Environ. Health.* 32:61-66.

Concentration of Use by FDA Product Category
2,4-Diaminophenoxyethanol HCl and 2,4-Diaminophenoxyethanol Sulfate

Ingredient	Product Category	Maximum Concentration of Use
2,4-Diaminophenoxyethanol HCl	Hair dyes and colors	0.56-2.4%
2,4-Diaminophenoxyethanol HCl	Hair lighteners with color	0.02%
2,4-Diaminophenoxyethanol HCl	Hair bleaches	0.00066%
2,4-Diaminophenoxyethanol Sulfate	Hair dyes and colors	0.25-0.35%

Information collected in 2022

Table prepared: October 28, 2022