Safety Assessment of m-Phenylenediamine and m-Phenylenediamine Sulfate as Used in Cosmetics
November 16, 2012

Memorandum

To: CIR Expert Panel
From: Wilbur Johnson, Jr.
Manager/Lead Specialist
Subject: Re-review Document on m-Phenylenediamine and m-Phenylenediamine Sulfate

A CIR final safety assessment on m-phenylenediamine and m-phenylenediamine sulfate with the following conclusion was issued at the August 28-29, 1995 Panel meeting and published in 1997: On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that m-phenylenediamine and m-phenylenediamine sulfate are safe for use in hair dyes at concentrations up to 10%. Literature searches on these 2 ingredients were performed to identify studies that entered the published literature since the final safety assessment was issued, and summaries of pertinent publications are included in this re-review document.

There is a second shoe, so to speak, with respect to this re-review. Self-testing for contact sensitization to hair dyes has come under some scrutiny in Europe. The suggestion seems to be that if the use of a hair dye self-test to predict contact sensitization becomes widespread, there is risk that there may be morbidity in European consumers. Two pieces of information are available to inform this part of the discussion; the report by Thyssen et al. in Contact Dermatitis and the editorial by An Goossens, also in Contact Dermatitis. In addition, we understand that the Council’s Hair Coloring Technical Committee will make a presentation at the meeting.

The re-review document on m-phenylenediamine and m-phenylenediamine sulfate is included along with the CIR report history, Literature search strategy, Ingredient Data profile, 2012 FDA VCRP data, Minutes from the March 16-17, 2005 (tentative report issued) and August 28-29, 1995 (final report issued) Expert Panel Meetings, the 1997 published CIR final safety assessment on m-phenylenediamine and m-phenylenediamine sulfate, use concentration data on these 2 hair dyes received from the Council (pdf data1 file), and 2 publications on self-testing for contact sensitization (pdf data 2 and data 3 files).

After evaluating this re-review document and accompanying materials, the Expert Panel needs to determine whether the CIR final safety assessment on m-phenylenediamine and m-phenylenediamine sulfate needs to be reopened.
# Safety Assessment Flow Chart

<table>
<thead>
<tr>
<th>Public Comment</th>
<th>CIR</th>
<th>Expert Panel</th>
<th>Re-Reviews</th>
<th>Report Color</th>
</tr>
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<tbody>
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<td></td>
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</table>

*The CIR Staff notifies of the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.

**If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.

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CIR Panel Book Page 1
CIR History of:

m-Phenylenediamine and m-Phenylenediamine Sulfate

A CIR final safety assessment on m-phenylenediamine and m-phenylenediamine sulfate with the following conclusion was issued at the August 28-29, 1995 Expert Panel meeting and published in 1997: On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that m-phenylenediamine and m-phenylenediamine sulfate are safe for use in hair dyes at concentrations up to 10%.

1st Re-review, Belsito and Marks Teams/Panel: December 10-11, 2012

Literature searches on these 2 ingredients were performed to identify studies that entered the published literature since the final safety assessment was issued, and summaries of pertinent publications are included in this re-review document. Unpublished use concentration data received from the Council have also been added.
### m-Phenylenediamine and m-Phenylenediamine Sulfate Check List for December, 2012.

**Analyst – Wilbur Johnson**

<table>
<thead>
<tr>
<th></th>
<th>Acute toxicity</th>
<th>Repeated dose toxicity</th>
<th>Irritation</th>
<th>Sensitization</th>
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</thead>
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<td>Oral</td>
<td>Parenteral</td>
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<td>m-Phenylenediamine Sulfate</td>
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</tr>
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</table>
Literature Searches on m-Phenylenediamine and m-Phenylenediamine Sulfate*

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Toxline &amp;PubMed</th>
<th>ChemIDplus</th>
<th>Multidatabase (See legend*)</th>
<th>DART</th>
<th>SciFinder</th>
<th>RTECS</th>
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*Data in Table: Publications found; Multidatabase = HSDB, CCRIS, ITER, IRIS, Gene-Tox, and LacMed

Searches (years1993-2012) Performed on 0920/2012

Ingredients/Search Terms

(m-phe) m-phenylenediamine
CAS No. 108-45-2

(m-phe S) m-phenylenediamine sulfate
CAS No. 541-70-8
CAS No. 25723-55-1
Day 2 of the August 28-29, 1995 CIR Expert Panel Meeting – Full Panel

**m-Phenylenediamine and m-Phenylenediamine Sulfate**

The Panel agreed that the reproductive toxicity and carcinogenicity studies on an epoxy resin hardener mixture containing m-Phenylenediamine should be deleted from the report text, because the results could not be interpreted in terms of the effects of m-Phenylenediamine alone. The Panel also approved editorial changes in the report discussion: In the first paragraph, the last sentence was edited to read as follows: Given these test results, the Expert Panel determined that m-Phenylenediamine and m-Phenylenediamine Sulfate can be used safely in hair dyes (hair dyes instead of cosmetics) at concentrations up to 10%. The last sentence of the second paragraph was edited to read as follows: However, the critical endpoints of carcinogenicity and teratogenicity were negative. The phrase, leading the Panel to dismiss the mutagenicity data, was deleted from the end of this statement.

Dr. Belsito noted that hair dye formulations containing 1.5% m-Phenylenediamine were tested in some of the dermal carcinogenicity studies (negative results) included in the CIR report, and questioned the Panel's approval of a concentration limit (10%) that is much higher than this test concentration. Based on the Panel's discussion on HC Orange No.1 earlier in the day, Dr. Belsito indicated that there may be inconsistencies relating to how the Panel establishes concentration limits based on the available data.

Dr. Slaga said that the Panel has not always been consistent in terms of what has been chosen to be the effect or the no-effect-level upon which a limitation is based. Opinions have varied from ingredient report to ingredient report, depending on which data were deemed strongest. He said that the data in this safety assessment support a 10% concentration limit for m-Phenylenediamine and m-Phenylenediamine Sulfate.

Dr. Shank commented that the issue of consistency as it relates to how concentration limits are established by the Panel needs to be resolved.

Dr. McEwen said that it should not be concluded that the carcinogenicity data on m-Phenylenediamine were ignored in the Panel's deliberations just because the concentration limit was not based on the test concentrations in carcinogenicity studies. He also said that in cases where there is no effect (e.g. negative carcinogenicity studies), the total exposure must be taken into consideration prior to establishing any concentration limit.

Dr. Jeffrey Yourick, with FDA, cautioned the Panel on the applicability of using a safety factor approach for looking at carcinogenicity.

The Panel voted in favor of issuing a Final Report on m-Phenylenediamine and m-Phenylenediamine Sulfate with the following conclusion: On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that m-Phenylenediamine and m-Phenylenediamine Sulfate are safe for use in hair dyes at concentrations of ≤ 10%.

Drs. Shank and Slaga abstained.

Given the present level of discussion prior to the vote, Dr. Bergfeld said that a discussion of the Panel's handling of data and the pivotal points that need to be qualified should be reviewed before issuance of the Final Report.
Day 2 of the March 16-17, 1995 CIR Expert Panel Meeting – Full Panel

**m-Phenylenediamine and m-Phenylenediamine Sulfate**

Dr. Bergfeld noted that the Draft Report on m-Phenylenediamine and m-Phenylenediamine Sulfate, reviewed in Teams on the preceding day, was added to today's agenda. This action was based on the Panel's determination that a Tentative Report could be issued at the present meeting.

Dr. Schroeter indicated that his Team was extremely pleased with the composition and comprehensive content of the Draft Report, and concluded that m-Phenylenediamine and m-Phenylenediamine Sulfate are safe as oxidative hair dyes at concentrations of $\leq 10\%$.

Dr. Belsito confirmed that the proposed concentration limit is based on skin sensitization data. He also asked whether, given dermal absorption, this concentration is consistent with the negative carcinogenicity studies that exist, and wanted to know whether this type of calculation had been made.

Dr. Shank said that, given the proposed maximum use concentration of 10% for use in oxidative hair dyes, the carcinogenicity test results have enabled the Schroeter Team to determine, with confidence, that there is no carcinogenic risk.

Dr. Belsito indicated that m-Phenylenediamine and m-Phenylenediamine Sulfate are used in cosmetics at concentrations up to 1%.

Dr. Schroeter said that the Panel arrives at conclusions based on the available toxicity data.

The Expert Panel concluded that m-Phenylenediamine and m-Phenylenediamine Sulfate are safe as oxidative hair dyes at concentrations of $\leq 10\%$ and voted unanimously in favor of issuing a Tentative Report.

Dr. Bergfeld said that a discussion that includes the following points should be added to the Tentative Report: (1) The reason for the 10% concentration limit (negative sensitization data) and (2) The consideration of mutagenicity, carcinogenicity, and teratogenicity test results in arriving at a safety determination.
Safety Assessment of m-Phenylenediamine and m-Phenylenediamine Sulfate as Used in Cosmetics

Status: Re-Review for CIR Expert Panel Review
Release Date: November 16, 2012
Panel Meeting Date: December 10-11, 2012

The 2012 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A. Hill, 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 Wilbur Johnson, Jr., M.S., Manager/Lead Specialist and Ivan Boyer, Ph.D., Toxicologist.
# Table of Contents

**INTRODUCTION** .......................................................................................................................................................................................... 1  
**CHEMISTRY** .......................................................................................................................................................................................... 1  
  - DEFINITION AND STRUCTURE .......................................................................................................................... 1  
  - CHEMICAL AND PHYSICAL PROPERTIES ................................................................................................. 2  
  - METHOD OF PRODUCTION ........................................................................................................................... 2  
**USE** ............................................................................................................................................................................................... 2  
  - COSMETIC .......................................................................................................................................................... 2  
**TOXICOLOGY** .................................................................................................................................................................................. 2  
  - OCULAR IRRITATION .................................................................................................................................. 2  
  - SKIN SENSITIZATION ................................................................................................................................. 3  
  - ENDOCRINE DISRUPTION ............................................................................................................................ 4  
**GENOTOXICITY** ................................................................................................................................................................................... 5  
  - MAMMALIAN ASSAYS .............................................................................................................................. 5  
  - BACTERIAL ASSAYS .............................................................................................................................. 5  
**CARCINOGENICITY** .............................................................................................................................................................................. 8  
  - INHIBITION OF METABOLIC COOPERATION .......................................................................................... 8  
  - SAR/QSAR MODELS ............................................................................................................................... 8  
**EPIDEMIOLOGY** .................................................................................................................................................................................... 8  
**SUMMARY FROM 1997 PUBLISHED CIR FINAL REPORT** .................................................................................................................. 8  
**DISCUSSION FROM 1997 PUBLISHED CIR FINAL REPORT** ........................................................................................................... 10
INTRODUCTION

A Cosmetic Ingredient Review (CIR) Final Report with the following conclusion was published 1997: On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that m-phenylenediamine and m-phenylenediaine sulfate are safe for use in hair dyes at concentrations of up to 10%.1 The summary and discussion from this final safety assessment are included at the end of this report. This re-review document includes data on m-phenylenediamine that either were not included in the Final Report or entered the published literature since its issuance. New data on m-phenylenediamine sulfate were not found in the published literature.

In the 1997 report, the CIR Expert Panel noted that hair dyes containing 2-Amino-6-Chloro-4-Nitrophenol and its hydrochloride salt, 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 cautionary statement and patch test instructions for determining whether the product causes skin irritation. To qualify for the exemption, the following caution statement must be displayed conspicuously on the labels of coal tar hair dyes:

Caution: This product contains ingredients that may cause skin irritation on certain individuals, and a preliminary test according to accompanying directions should be made.

CIR has further elaborated that these preliminary test 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.

Earlier this year, Thyssen et al. published a report regarding such self-testing for contact sensitization to hair dyes.2 These authors concluded that, in its present form, the hair dye self-test has severe limitations. The authors issued the warning that, if the use of a hair dye self-test to predict contact sensitization becomes widespread, there is severe risk that a tool has been marketed that may cause morbidity in European consumers. In accompanying editorial,3 An Goossens, on behalf of the European Society of Contact Dermatitis, asserted that industry is focusing on predicting the risks from exposure to hair dyes by having millions of European consumers perform a self-test prior to each hair dying and stated that it is the opinion of the ESCD that attention must be given to reducing the risks of serious allergic reactions by improving the safety of the products themselves.

CHEMISTRY

Definition and Structure

m-Phenylenediamine (CAS No. 108-45-2) is defined as the aromatic amine that conforms to the following structural formula4:

Other names for this chemical include: m-aminoaniline; 1,3-benzenediamine; CI 76025; Developer 11; 1,3-diaminobenzene; and 1,3-phenylenediamine.

m-phenylenediamine sulfate (CAS Nos. 25723-55-1 and 541-70-8) is defined as the aromatic amine salt that conforms to the following structural formula4:
Other names for this chemical include 1,3-benzenediamine sulfate and 1,3-phenylenediamine sulfate.

**Chemical and Physical Properties**

An octanol-water partition coefficient (log P) of 0.34 has been reported for m-phenylenediamine. m-Phenylenediamine has a UV absorption maximum at approximately 220 nm.

**Method of Production**

The synthesis of m-phenylenediamine from m-dinitrobenzene over silica-supported nickel catalyst has been reported. The liquid-phase hydrogenation of m-dinitrobenzene was carried out in a stainless steel autoclave at 373 K and 2.6 MPa hydrogen pressure. m-Dinitrobenzene (2.72 g) was dissolved in 100 ml ethanol. Silica-supported nickel catalyst (0.272 g) was loaded into the autoclave vessel.

**USE**

**Cosmetic**

Both m-phenylenediamine and m-phenylenediamine sulfate function as hair colorants. According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP) in 2012, m-phenylenediamine was being used in 46 hair dyes and colors and m-phenylenediamine sulfate was being used in 19 hair dyes and colors. Results from a survey of ingredient use concentrations provided by the Personal Care Products Council in 2012 indicate that m-phenylenediamine was reported as being used at maximum concentrations ranging from 0.01% to 0.2%, and, m-phenylenediamine sulfate, at a maximum concentration of 1% in hair dyes and colors.

Permanent hair coloring formulations containing m-phenylenediamine and m-phenylenediamine sulfate are applied to, or may come in contact with, hair, skin (particularly the scalp), eyes, and nails. Individuals dyeing their hair may use such formulations once every few weeks, whereas hairdressers may come in contact with products containing m-phenylenediamine and m-phenylenediamine sulfate several times per day.

According to the European Union Cosmetics Directive, m-phenylenediamine and its salts are among the substances which must not form part of the composition of cosmetic products marketed in the European Union.

**TOXICOLOGY**

**Ocular Irritation**

The ocular irritation potential of m-phenylenediamine was predicted using the EYETEX™* in vitro* test system at 7 different laboratories. Results were compared with results from the Draize test. The EYETEX test is based on the contribution of protein aggregation to the production of corneal opacity. The synthetic protein matrix of the EYETEX reagent was developed to produce opacity after exposure to chemical irritants in the same manner that the cornea produces opacity *in vivo*. Undiluted m-phenylenediamine and m-phenylenediamine (10% w/v or 1% w/v in distilled water) were tested at the following volumes: 10, 20, 30, or 100 µl. The samples were incubated for 24 h. A set of calibrators defined the
relationship of the optical density produced in the reagent to the Draize score. Optical density readings were converted to an EYETEX/Draize equivalent, and irritancy class was recorded.

EYETEST results classified m-phenylenediamine (100%) as a severe ocular irritant, m-phenylelenediamine (10%) as a moderate ocular irritant, and 1% m-phenylenediamine as a minimal ocular irritant. Draize test results on m-phenylenediamine (100%, as powder) and m-phenylenediamine (10% suspension) were also presented. The maximum average Draize scores were 80.7 (undiluted m-phenylenediamine, severe ocular irritant) and 4.3 (10% m-phenylenediamine suspension, mild ocular irritant).11

A new alternative method to evaluate the ocular irritation potential of chemicals was performed using a human cornea cell line (HCE-T cells).12 HCE-T cells were exposed to different concentrations of m-phenylenediamine (serial dilutions: 0.078% to 20%; solvent = medium containing 5% DMSO) and numerous other chemicals individually for 1 h, and relative cell viability (RCV) was measured as an endpoint at each concentration. Medium RCV values were used to calculate the RCV50 for each of the 3 experiments. HCE-T cells were also exposed to 3 fixed concentrations of the 38 chemicals (5%, 0.5%, and 0.05%) for 1 h, and the RCV was measured at each concentration. The RCV values at 5%, 0.5%, and 0.05% were used to develop a new criterion for ocular irritation potential (total eye irritation score [TEIS]), and ocular irritation was estimated. Next, the correlation of the RCV50 and TEIS with Draize ocular irritation test (rabbits) results was assessed.

For the 3 serial dilution experiments involving HCE-T cells, RCV50 values of 5472 µg/ml (irritant), 4765 µg/ml (irritant), and 4800 µg/ml (irritant) were reported. TEIS scores of 10 (irritant), 13 (irritant), and 10 (irritant) for the 3 experiments were reported. m-Phenylenediamine was classified as an ocular irritant in the in vivo Draize ocular irritation test (rabbits). A modified maximum average score (MMAS) score of 80.7 was reported for undiluted m-phenylenediamine, and an MMAS of 4.3 was reported for 1% m-phenylenediamine. The authors noted that both the RCV50 and TEIS results for the 38 chemicals evaluated showed good positive correlations (sensitivity: 80.77%, specificity: 83.33%, and accuracy: 81.58% for TEIS; sensitivity: 73.08-76.92%, specificity: 75.0%, and accuracy: 73.68-76.32% for RCV50). It was concluded that the new in vitro model using HCE-T cells is a good alternative evaluation model for the prediction of the ocular irritation potential of chemicals.12

Skin Sensitization

Animal

The skin sensitization potential of m-phenylenediamine was studied using groups of 5 CBA/J female mice (6 weeks old), provided with feed supplemented with vitamin A acetate (known to boost response to contact allergens) for 3 weeks and throughout the duration of the experiment.13 CBA/J spleen cells were incubated with m-phenylenediamine (3 µg/ml) in petri dishes for 18 h. Adherent spleen cells were then scraped off and combined with non-adherent cells, and comprised the antigen pulsed spleen cells that would be injected. At the end of the 3-week feeding period, the mice were sensitized by intraperitoneal (i.p.) injection of allergen-pulsed spleen cells. Three injections were made at 1-week intervals. At 10 days after the last injection, the mice were challenged. During challenge, right ears were painted with 1% m-phenylenediamine in hydroxycellulose vehicle. The left ear was painted with vehicle. Ear swelling was calculated as the difference in ear thickness from the initial measurement. Naive mice were challenged as a control for irritant induced ear swelling. Good ear swelling was induced by 1% m-phenylenediamine: 5.5 mm (at 24 h), 7.0 mm (at 48 h), and 4.0 mm (at 72 h). Naive mice did not exhibit ear swelling.

In Vivo/In Vitro

Cross-sensitization between p-phenylenediamine and p-aminophenol or m-phenylenediamine was evaluated using a modified lymphocyte transformation test.14 Initially, 2 groups of female Hartley Guinea pigs (experimental [9 animals]; controls [5 animals]) were treated with p-phenylenediamine (sensitizer) and distilled water, respectively, according to the maximization test procedure. On day 1, two rows of 3 injections were given simultaneously to every guinea pig in the experimental group as follows: (1) 0.1 ml 0.1% p-phenylenediamine in distilled water; (2) 0.1 ml Freund’s complete adjuvant; (3) 0.1 ml p-phenylenediamine emulsified in Freund’s complete adjuvant at 0.1%. Seven days later, 0.5 ml 5% p-phenylenediamine in distilled water was spread over a 2 x 4 cm patch of filter paper. The patches were secured with impermeable adhesive tape, which was removed 48 h later. The control group was subjected to the same procedure, using distilled water and Freund’s complete adjuvant. Using a Finn chamber with a closed patch (24-h application), both the experimental and control groups were challenged in vivo 21 days after injections with 0.025 ml 1% p-phenylenediamine, 1% p-aminophenol, and 5% m-phenylenediamine in distilled water. Reactions were scored at 24 h post-removal of the Finn chamber according to the International Contact Dermatitis Research (ICDRG) grading scale (negative to extreme positive). Responses to 5% m-phenylenediamine at challenge could not be read due to skin pigmentation.
The animals were then killed and lymph node cells of sensitized (n = 9/group) or non-sensitized (n = 5/group) were prepared from peripheral lymph nodes. The lymph node cells from sensitized or control guinea pigs were then placed in microplates (5 x 10^5 cells/well), after which the following were added: epithelial cells (10^5 cells/well) and m-phenylenediamine (0, 1, and 5 ppm), p-aminophenol (0, 1, and 5 ppm), or m-phenylenediamine (0, 5, and 25 ppm). [The results of a preliminary cytotoxicity test indicated that m-Phenylenediamine was toxic to lymphocytes at concentrations ≥ 50 ppm; thus, the maximum concentration for in vitro challenge was 25 ppm.] The same series of preparations was incubated without epithelial cells. As the positive control for lymphocyte proliferation, phytohemagglutinin (1 µg/well) was added instead of antigens. Cells were then incubated with [3H]-thymidine for 24 h and subsequently harvested, at which time the level of [3H]-thymidine incorporation was determined. The results of a preliminary cytotoxicity test indicated that m-Phenylenediamine was toxic to lymphocytes at concentrations ≥ 50 ppm; thus, the maximum concentration for in vitro challenge was 25 ppm.

When the amount of [3H]-thymidine incorporated into lymphocytes (measurement of blastogenesis) from p-phenylenediamine-sensitized and control animals stimulated with m-phenylenediamine was evaluated, the difference between 0 ppm and 25 ppm m-phenylenediamine was statistically significant (p < 0.01). Blastogenesis in lymphocytes from sensitized guinea pigs was enhanced when these cells were cultured with p-phenylenediamine, p-aminophenol, or m-phenylenediamine in the absence or presence of epidermal cells than without the sensitziers. Furthermore, the extent of the response depended on the concentration of p-phenylenediamine, p-aminophenol, or m-phenylenediamine added to the cultures. Blastogenesis in lymphocytes from control animals was not significantly enhanced in response to p-phenylenediamine, p-aminophenol, or m-phenylenediamine in the presence or absence of epidermal cells. The extent of the responses was on the order of p-phenylenediamine > p-aminophenol > m-phenylenediamine. In contrast, because p-phenylenediamine, p-aminophenol, and m-phenylenediamine are color developing agents, cross-sensitization between p-phenylenediamine and p-aminophenol or m-phenylenediamine could not be evaluated by the results of an in vivo challenge, due to pigmentation in the patch application sites. Study results suggested that there is cross-sensitization between p-phenylenediamine, p-aminophenol, or m-phenylenediamine, and that the modified lymphocyte transformation test is a useful predictive means of detecting cross-sensitization among chemicals, especially for color developing agents.

The skin sensitization potential of m-phenylenediamine was evaluated using the local lymph node assay. The induction phase of skin sensitization is associated with and dependent upon the initiation of T lymphocyte responses in lymph nodes draining the site of exposure. Such responses are characterized by lymph node cell hyperplasia, and it is this event that is measured in the local lymph node assay. Groups of mice (number and strain not stated) were exposed (on dorsum of both ears) daily for 3 consecutive days to various concentrations of m-phenylenediamine (2.5%, 5%, and 10%). At 5 days after initiation of exposure, the mice were injected intravenously (i.v.) with [3H]thymidine and activity was measured as a function of isotope incorporation in draining auricular lymph nodes. Potential sensitizers are classified as chemicals, at least one concentration, that provoke a 3-fold or greater increase in isotope incorporation (i.e., proliferative activity), when compared to the vehicle-treated controls. Chemicals that fail to stimulate such responses are regarded as not possessing significant sensitization potential. m-Phenylenediamine was classified as active in the local lymph node assay.

**QSAR Model**

The skin sensitization potential of m-phenylenediamine and 228 other hair dye substances registered in Europe was estimated using a quantitative structure-activity relationship (QSAR) model. This model was developed using experimental local lymph node assay data and topological sub-structural descriptors (TOPS-MODE). The model categorizes potency into 1 of the following 3 bands: strong/moderate, weak, or extremely weak/non-sensitizing. The classifications of strong, moderate, and weak are based on studies in which LLNA potency data have been compared and have been correlated with human potency information. m-Phenylenediamine was predicted to be a strong/moderate sensitizer, with a predicted sensitization potency value of 1.8.

**Endocrine Disruption**

A study was performed to provide insight into the use of QSAR models to address endocrine disruption effects mediated through the estrogen receptor (ER). New predictive models were derived to assess estrogenicity for a very large and heterogeneous dataset of chemicals (~1000 compounds). The focus was on multiple endpoints to better characterize the effects of endocrine disrupters, evaluating both receptor binding (RBA) and reporter gene (RA) transcriptional activity. A possible combination of the RBA and RA models was also explored. The source of activity data was the Japanese Ministry of Economy Trade and Industry (METI) database, one of the largest collections of data (on > 900 compounds) for the ER publicly available. It contains experimentally determined values of human ERα for both RBA and RA assays, expressed as
percentage of activity, using 17β-estradiol as the reference. The dataset collected in this study was divided into the following 3 parts: (1) the training set constituted by the examples provided to the learning algorithms; 2) the validation set used to assess which are the best parameters and architecture for the models; and (3) a test set to independently assess a group of compounds (never used before) to assess the model’s performances in prediction. Very good accuracy was achieved with both RA and RBA models (> 80%). In both RBA and RA assays, m-phenylenediamine was classified as inactive, i.e., no endocrine disruption activity.

GENOTOXICITY

Genotoxicity study summaries are included in Table 1.

Mammalian Assays

The ability of m-phenylenediamine (in deionized water) to induce micronuclei in bone marrow polychromatic erythrocytes was evaluated using groups of 6 male and female CrI:CD®-1(ICR)BR mice (50 days old; 3 males, 3 females per group). The test substance was administered orally (intubation) to 3 groups in doses of 16, 33, or 65 mg/kg body weight. Each group received 2 doses, separated by 24 h. The negative control group was dosed with deionized water (10 ml/kg), and the positive control group was dosed with cyclophosphamide (20 mg/kg). For the vehicle and 65 mg/kg/day groups, bone marrow smears were prepared approximately 24 and 48 h after the final dose. For the 16 and 33 mg/kg/day groups, bone marrow smears were prepared 24 h after the final dose. One thousand polychromatic erythrocytes (PCEs) per animal were evaluated for the presence of micronuclei.

No statistically significant increases in the frequency of micronucleated PCEs were observed in mice of any group dosed with m-phenylenediamine. A significant depression in the ratio of young PCEs to mature normochromatic erythrocytes was observed in high-dose males at the 48-h sampling interval. It was concluded that, under the conditions of this assay, m-phenylenediamine did not induce micronuclei.

The genotoxicity of m-phenylenediamine was evaluated using Chinese hamster ovary cells (CHO-K1). Eagle’s basal medium and DMSO served as negative controls, and triethylenemelamine (TEM) served as the positive control. Cultures were incubated in Eagle’s basal medium for 2h with concentrations of m-phenylenediamine (in DMSO) up to 9363 µg/ml. Both the percentage of aberrant cells (number of cells with structural aberrations per 100 cells) and the frequency of aberrations (total number of aberrations per 100 cells) were calculated. m-Phenylenediamine induced a positive dose-related increase in chromosomal aberrations. Gaps, breaks, deletions, exchanges, and dicentrics were present. The positive control was also genotoxic.

The genotoxicity of m-phenylenediamine was evaluated in the comet assay. Human lymphocytes were incubated with m-phenylenediamine (0.5 to 25 mM) for 4 h with or without metabolic activation (TX1MX plant activation mix). Without activation, m-phenylenediamine induced DNA strand breaks, alkali-labile sites, and incomplete excision repair sites in human lymphocytes. In the 0.5 to 25 mM concentration range, a positive effect on the induction of increasing mean tail moment values was detected. At concentrations ≥ 7.5 mM, significantly larger mean tail moment values were induced (P < 0.001), compared to negative control values. With activation, a dramatic reduction in the genotoxicity of m-phenylenediamine to human lymphocytes was observed. Though m-phenylenediamine concentrations > 1 mM + TX1MX significantly increased the mean tail moment values (P ≤ 0.001), this increase did not approach the levels of DNA damage induced by m-phenylenediamine alone.

Bacterial Assays

The genotoxicity of m-phenylenediamine in Salmonella typhimurium strain YG 1024, with and without plant metabolic activation (TX1MX plant activation mix), was studied. TX1MX is a cell-free plant activating mixture prepared from Nicotiana tabacum cells (line TX1). The bacterial strain was incubated for 1 h with m-phenylenediamine (10 µM to 5 mM). Toxicity was not observed. Without activation, m-phenylenediamine did not significantly increase the mutant yield (P = 0.602). With activation, a significant concentration-response over the negative control was observed (P ≤ 0.001).

The genotoxicity of m-phenylenediamine, without and with metabolic activation (S9 mix), was evaluated using Salmonella typhimurium strains TA 98 and TA100. m-phenylenediamine (in DMSO) was evaluated at doses up to 3000 µg/plate. DMSO served as the negative control and the positive controls were: 2-nitrofluorene (strain TA98 without activation), N-methyl-N’-nitrosoguanidine (MNNG, strain TA100 without activation), and 2-aminofluorene (strains TA98...
and TA100 with activation). With metabolic activation, m-phenylenediamine was highly genotoxic to strain TA98, but weakly genotoxic to strain TA100. Results were negative without metabolic activation.

The genotoxicity of m-phenylenediamine HCl (in dimethylsulfoxide [DMSO]) was evaluated using *Salmonella typhimurium* strains TA 98 AND TA 100.\textsuperscript{21} The test substance was evaluated at concentrations up to 1078 µg/plate with (S9 mix) and without metabolic activation. DMSO served as the negative control. 2-Aminoanthracene served as the positive control for strains TA 98 and TA 100 with metabolic activation. 2-nitrofluorene served as the positive control for strain TA 98 without metabolic activation, and sodium azide served as the positive control for strain TA 100 without metabolic activation. Results were positive for m-phenylenediamine, only in strain TA 98 with metabolic activation. All positive controls were genotoxic.

A study was performed to determine whether enzymes secreted in tobacco suspension culture medium could activate m-phenylenediamine, and to quantify the concentration-response relationship by the active medium as a function of cell growth phase, protein concentration, and peroxidase activity.\textsuperscript{22} The plant cell/microbe coincubation assay was performed according to minor modifications of the procedure by Wagner et al.\textsuperscript{23} *Salmonella typhimurium* strains YG1024, TA98, and TA98/1,8-DNP\textsubscript{6} and cell suspension cultures of *Nicotiana tabacum* line TX1 were used. Each reaction tube contained 250 µl of bacterial suspension, 2 ml of 100 mg/ml tobacco cells, a known concentration of m-phenylenediamine (0.5 to 2 mM), and 50 mM citrate phosphate buffer (pH 6.5). The reaction tubes were incubated for 1 h at 28°C. Concurrent negative controls included tobacco cells and bacterial cells alone. In a second experiment, cell free activation medium (TX1MX) was prepared by removing TX1 cells from the medium. Each reaction tube consisted of 250 µl of bacterial suspension, 2 ml of TX1MX, m-phenylenediamine (100 µM to 2 mM), and 5 mM citrate-phosphate buffer (pH 6.5). The reminder of test procedure was the same as that of the plant/cell microbe coincubation assay.

Over the concentration range of 100 µM to 2 mM m-phenylenediamine, TX1MX was more effective than intact TX1 cells in activating m-phenylenediamine to a product that was mutagenic to *Salmonella typhimurium* strains TA98 and YG1024. Mutagenicity increased with increasing p-phenylenediamine concentrations in TX1MX cell-free activation medium. There was no evidence of genotoxicity in strain TA98/1,8-DNP\textsubscript{6}. Additionally, data presented in this study suggest that the peroxidases present in the recovered cell culture medium or in the high molecular weight matrix are responsible for the plant activation of m-phenylenediamine.\textsuperscript{22}

The ability of the medium recovered from tobacco cell suspensions (TX1MX, contains peroxidases) to activate 1 mM m-phenylenediamine (in potassium phosphate buffer) into products (names not provided) mutagenic to *Salmonella typhimurium* strain YG1024 was determined for samples isolated from day 2 to day 14 *Nicotina tabacum* TX1 cell suspension cultures.\textsuperscript{24} Experiments were performed according to the methods described in Plewa et al.\textsuperscript{25} TX1MX expressed an increased capacity to activate m-phenylenediamine from day 2 to day 8, and this activation reached a plateau after day 8. TX1MX supernatants expressed an increased capacity to activate m-phenylenediamine, which paralleled the response of the TX1MX From Day 2 to day 9. However, TX1MX supernatants from day 10 to day 14 had a significantly lower ability to activate m-phenylenediamine than the TX1MX samples from which they were prepared. A 25% dilution (w/v) of the gel activated m-phenylenediamine to a great degree. This activaton capacity remained at a high level, while the capacity to activate m-phenylenediamine was lost from the corresponding supernatant fractions. The gel fraction isolated from TX1MX was more potent in activating m-phenylenediamine into a mutagenic product than TX1MX or the supernatant fraction.\textsuperscript{24}

A study was performed to assess whether m-phenylenediamine induced mutations in intact cells of *Chlamydomonas reinhardtii* (alga wild-type strain W1 and repair-deficient strains uvs10, uvs12, and uvs14) and *Saccharomyces cerevisiae* strain D7 (yeast).\textsuperscript{26} Whether m-phenylenediamine was activated to a mutagen in algal cell/microbe coincubation assays was also determined using *Salmonella typhimurium* strains TA98 and YG1024 (TA98 derivatives with elevated O-acetyltransferase activity) and *Saccharomyces cerevisiae* strain D4. Assay procedures and results are summarized below.

In the intact algal cells toxicity and mutagenicity assays, all *Chlamydomonas reinhardtii* strains were treated for 1 h with different concentrations of m-phenylenediamine in phosphate buffer (≤ 25 mM to between 250 and 300 mM). Streptomycin-resistant mutants induced by m-phenylenediamine were isolated, and the frequency of mutations was expressed as the number of colonies formed in the presence of 100 µg/ml streptomycin/10⁶ surviving cells. The recombination-repair-deficient strain (uvs10) was most sensitive to treatment, while the excision-repair-deficient strain (uvs12) was the most resistant one. m-Phenylenediamine was toxic at concentrations greater than 250 mM. Results for m-phenylenediamine mutagenicity indicated that the highest rate of streptomycin-resistant mutants was found in strain uvs14 (strain with higher level of spontaneous and induced mutability). The excision-repair deficient strain uvs12 did not mutate at all.\textsuperscript{26}

In the intact yeast cells (*Saccharomyces cerevisiae* strain D7), toxicity and mutagenicity assays, exponentially growing yeast cells were treated with different concentrations of m-phenylenediamine in phosphate buffer (1, 2, and 4 mM)
and plates were incubated at 28°C for 24 h. Survival and genetic changes were evaluated after 5 to 12 days. The toxic effect was dose-dependent. Mitotic crossing over was observed, but only at a very low level. The frequency of total aberrants of the ade locus was low and approximately the same at all test concentrations. The frequency of convertants per 10⁵ survivors was increased, and, at the highest test concentration, was 10-fold higher when compared to the control (not stated, but 100% survival noted for control). The frequency of revertants per 10⁶ survivors was elevated at the highest concentration, and was ~7- to 8-fold over the control.²⁶

In the algal cell/Salmonella typhimurium coinubcation assay, both algal and bacterial cells were treated for 48 h with m-phenylenediamine (0.5 to 60 mM). Plates were incubated for 72 h at 37°C and revertant His+ colonies were counted. The potential toxic effect of m-phenylenediamine was measured using the spot test; bacterial survival was assessed. At low concentrations (0.5 to 5 mM), m-phenylenediamine did not induce His+ revertants at the short period (1 h) of activation in any experimental variant using strains TA98 and YG1024. However, differences between indicator strains were noted after treatment with higher concentrations of m-phenylenediamine (30 to 60 mM). At higher concentrations, the number of revertants was increased 2.5 fold only in strain YG1024. Neither toxicity nor changes in survival were observed over the range of concentrations tested. The preceding assay was followed by the cell-free activation medium/bacteria (Salmonella typhimurium) assay. In this assay, algal cells were removed from the medium by centrifugation. Each reaction tube contained bacterial suspension, varying concentrations of m-phenylenediamine (0.5 to 60 mM), and cell-free activation medium. The remainder of the procedure was identical to that described for the preceding algal cell/Salmonella typhimurium coinubcation assay. The results reported for this assay were similar to those reported for the algal cell/Salmonella typhimurium coinubcation assay.²⁶

Two variants of the algal cell/Saccharomyces cerevisiae coinubcation assay were also used, the algal supernatant/yeast assay and the cell free activation medium/yeast assay. In the algal supernatant/yeast assay, algae (wild-type of C. reinhardtii) were treated with different concentrations of m-phenylenediamine (1, 2, 4, or 8 mM). After 3 h of treatment at 26°C, the algal culture was centrifuged and the supernatant was used for 24-h treatment of exponentially growing Saccharomyces cerevisiae yeast of the D7 strain. The frequency of convertants at the highest test concentration (8 mM) was increased by 15-fold, when compared to the control with algal supernatant, and by 1.5- fold over that in intact yeast cells. The frequency of revertants at the highest concentration (8 mM) was elevated 17-fold, compared to the control. When the frequency of revertants in the experimental variant with algal supernatant was compared with that in intact cells, a 2.5-fold increase of mutations in the activated variant was reported. A 2-fold increase in the absolute number of revertants was also found in this variant.²⁶

In the cell free activation medium/yeast assay, algal culture was centrifuged and the recovered cell free activation medium was used for activation of m-phenylenediamine that was applied to yeast cells for 24 h at different test concentrations (2, 4, 8, 16, or 20 mM). Both toxicity and mutagenicity were evaluated according to the procedures used in the algal cell/Salmonella typhimurium coinubcation assay mentioned above. The m-phenylenediamine toxic effect in the cell free activation medium/yeast assay was lower when compared with its toxic effect on intact yeast cells or in the coinubcation assay with supernatant. Due to these findings, higher concentrations of m-phenylenediamine had to be used to achieve the same toxic effect. At equitoxic concentrations, the frequencies of genetic changes induced by m-phenylenediamine were very similar to those observed after m-phenylenediamine treatment of intact yeast cells, and the mutagenic effect of m-phenylenediamine was lower when compared with algal supernatant activation.²⁶

The preceding results of this study indicate that m-phenylenediamine induced mutations in intact cells of Chlamydomonas reinhardtii and Saccharomyces cerevisiae, and that m-phenylenediamine was activated to a mutagen in algal cell/microbe coinubcation assays using Salmonella typhimurium strains TA98 and YG1024 and Saccharomyces cerevisiae strain D4.²⁶

The extent of DNA damage induced by m-phenylenediamine in the presence of Cu(II) was studied using ³²P-5'-end labeled DNA fragments obtained from the human c-Ha-ras-1 protooncogene and the p53 tumor suppressor gene.²⁵ The reaction mixture contained the 32P-5'-end labeled 261-base pair DNA fragment, 20 μM/base of sonicated calf thymus DNA, m-phenylenediamine (50, 100, 200, or 500 μM) and CuCl₂ in phosphate buffer containing diethylenetriamine-N,N',N",N"-pentaacetic acid (DTPA). The mixture was incubated for 2 h at 37°C. DNA fragments were then treated with piperidine, electrophoresed, and autoradiograms were obtained. m-Phenylenediamine did not cause clear DNA damage or significant autoxidation at either of the 4 test concentrations, even in the presence of Cu(II).
CARCINOGENICITY

Inhibition of Metabolic Cooperation

The potential for inhibition of metabolic cooperation, a feature associated with the carcinogenic process, was evaluated according to the method of Budanova et al. Details relating to this test procedure (including test concentrations) are not presented in the present study. Results were negative for m-phenylenediamine.

SAR/QSAR Models

The OECD (Q)SAR Application Toolbox and Toxtree software tools include different SAR and QSAR models for estimating (eco)toxicological endpoints. One such model, the Benigni/Bossa rule-based system, is proposed to characterize the carcinogenic potential of chemicals. The current study evaluates the predictive performance that can be expected from the OECD (Q) SAR Toolbox and Toxtree when analyzing chemicals by means of the structural alerts coded within the Benigni/Bossa rule-based system for carcinogenicity and the associated QSAR model (QSAR8). In this report, a chemical was considered carcinogenic if it induced tumors in at least one rodent species (rats or mice, if it was carcinogenic according to RTECS criteria, or if it belonged to groups 1 or 2A or 2B of the IARC classification. Regarding a chemical comprising several structural fragments, each of which is within the domain of the QSAR model of the rule-based system, an overall prediction was considered negative only if all of the individual predictions were negative. However, a single positive prediction was sufficient to categorize the chemical as carcinogenic. Results for m-phenylenediamine and many other chemicals are presented in this report. Based on experimental data, m-phenylenediamine was negative for carcinogenicity. However, using both QSAR Toxtree and Toolbox software, m-phenylenediamine was classified as positive for carcinogenicity.

The authors noted that, overall, the findings in this study confirm the performance of the system of structural alerts, while suggesting that the sensitivity of QSAR8, as implemented in the 2 tools, is lower than what was previously reported. It was also stated that attention has to be paid when interpreting the output of the 2 tools because of possible malfunctions involving the coding of two-dimensional structures. A set of possible modulating factors for the structural alert identifying polycyclic aromatic hydrocarbons was also proposed, together with candidates for putative new structural alerts not included in the tested tools.

EPIDEMIOLOGY

m-Phenylenediamine and m-phenylenediamine sulfate are oxidative hair dye ingredients. 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. Currently available epidemiology studies provided insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. A detailed summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/cir-findings.

SUMMARY FROM 1997 PUBLISHED CIR FINAL REPORT

Both m-phenylenediamine and m-phenylenediamine sulfate are aromatic amines. Their molecular weights are 108.15 and 206.21, respectively. m-Phenylenediamine is soluble in alcohol, ether, and water and is produced by the reduction of metadinitrobenzene or nitroaniline with iron and hydrochloric acid. The sulfate may be prepared by reaction of the appropriate amine with sulfuric acid.

m-Phenylenediamine and m-phenylenediamine sulfate are used as hair colorants in 162 and 28 hair dye products, respectively. Both ingredients have been used in hair dyes at concentrations of up to 1%. Data submitted to the Personal Care Products Council (then Cosmetics, Toiletries, and Fragrance Association [CTFA] in 1995 indicated that m-phenylenediamine and m-phenylenediamine sulfate were used in hair colors at concentrations of up to 3%.

The percutaneous absorption of m-phenylenediamine has been demonstrated in dogs (hydrochloride salt in gel) and rats (14C- m-phenylenediamine in saline). The principal route of excretion of percutaneously absorbed m-phenylenediamine
in rats was in the urine; the following three urinary metabolites were also identified: N-acetyl-1,3-diaminobenzene, N,N'-diacetyl-2,4-diaminophenol, and N,N'-diacetyl-1,3-diaminobenzene. m-Phenylenediamine has also been detected in the urine of humans following ingestion of m-phenylenediamine hydrochloride.

Nine of 10 rats exposed (inhalation) to m-phenylenediamine distilled flakes for 6 h died within 24 h to 48 h after the end of exposure. Pulmonary congestion was noted in all 10 animals at necropsy. In another study, the mean acute inhalation IC$_{50}$ (groups of 10 rats, 4-h exposure) for m-phenylenediamine was 3.2 mg/L.

The acute oral LD$_{50}$ for m-phenylenediamine (in oil-in-water emulsion) in a group of 10 rats was 650 mg/kg. In other tests involving m-phenylenediamine distilled flakes (20% aqueous solution), the acute oral LD$_{50}$ in groups of five rats was 360 mg/kg, and the minimal lethal dose in a group of six rabbits was in the range of 250 mg/kg to 500 mg/kg.

Minimal nephrosis was the only pathologic change noted in six rats dosed orally with m-phenylenediamine (3% suspension in peanut oil; dose = 90 mg/kg/day) over a 2-week period. There were no test substance-related pathologic changes in six rats dosed (intragastric intubation) with 0.8% m-phenylenediamine (in mixture; 1354 mg/kg/day) over a 2-week period.

In a subchronic (90-day) oral toxicity study involving groups of 20 rats, the no-effect level was 6 mg m-phenylenediamine/kg body weight. At histopathologic examination, degenerative lesions in the liver were observed only in the 18-mg/kg/day dose group. There was no indication of toxic injury to the kidneys.

The dermal application of undiluted m-phenylenediamine to two pairs of rabbits (doses of 200 mg/kg and 2000 mg/kg, respectively) did not result in death, and the test substance was classified as nontoxic. At necropsy, hepatorenal toxicity (pale and swollen livers and kidneys) was noted in animals from groups of five mice that received daily applications of m-phenylenediamine in acetone. Concentrations of 10% and greater were applied over a 2-week period.

There was no evidence of test substance-induced toxicity in 12 rabbits that received dermal applications (1 mL/kg) of an oxidative dye formulation containing 1.5% m-phenylenediamine for 13 weeks. The dye was mixed with an equal volume of hydrogen peroxide prior to application.

At most, m-phenylenediamine was classified as a moderate ocular irritant in albino rabbits. m-Phenylenediamine (as supplied) was classified as a corrosive material in rabbits; however, in another study, undiluted m-phenylenediamine was not a primary skin irritant.

m-Phenylenediamine (25% and 35% aqueous solutions) induced irritation and sensitization reactions in albino guinea pigs. When guinea pigs were tested with lower concentrations of m-phenylenediamine (0.1% to 10.0%), skin irritation was noted at the highest concentration. Mild and no sensitization were noted at concentrations of 1.0% and 0.1%, respectively. There was no evidence of cross-sensitization in guinea pigs sensitized with p-phenylenediamine and challenged with 1% m-phenylenediamine in another study.

m-Phenylenediamine was not neurotoxic when administered orally to rats at doses of up to 20 mg/kg for 90 days.

When administered orally to female rats at doses of up to 90 mg/kg/day on gestation days 5 to 16, m-phenylenediamine was strongly fetotoxic at the highest dose, but not teratogenic at any of the doses tested. m-Phenylenediamine also was not teratogenic when administered on gestation days 6 to 15 at doses of up to 180 mg/kg/day. Oxidative hair dye formulations containing 1.5% m-phenylenediamine were not teratogenic in rats or mice.

In most of the Ames mutagenicity assays, m-phenylenediamine was mutagenic to Salmonella typhimurium strains with, but not without, metabolic activation. In human lymphocyte cultures, m-phenylenediamine was classified as a borderline mutagen in the chromosomal aberrations assay and was not mutagenic in the cytogenetics assay (with or without metabolic activation). Positive and negative responses were also noted in a variety of other in vitro and in vivo mutagenicity tests.

In studies involving mice and rats, neither m-phenylenediamine nor hair dye formulations containing this ingredient were carcinogenic. m-Phenylenediamine was tested in oral and dermal carcinogenicity studies, whereas the hair dye formulations were tested only in dermal carcinogenicity studies. Fibrosarcomas were observed in one of five rats injected subcutaneously with m-phenylenediamine over a period of 5 months; tumors were not observed in a second group dosed with 9 mg/kg.
m-Phenylenediamine-induced sensitization was noted in 1 of 15 patients. Four of the subjects were sensitive to m-phenylenediamine and p-phenylenediamine.

Positive scratch test results were reported for 8% of the workers (number not stated) who came in contact with m-phenylenediamine during its production over a period of 5 to 10 years. In another study, 8 of 38 dermatitis patients who had been exposed to m-phenylenediamine in the workplace had sensitization reactions to this ingredient. The 8 patients with positive reactions and the remaining 22 (negative reactions) had been exposed over periods of 20 and 14.5 months, respectively.

Hyperreflexia, hyporeflexia, skin hyperesthesia, and pathologic changes in the kidneys and liver were observed in humans (number not stated) professionally exposed to m-phenylenediamine at doses of 1 to 2 µg/L. The duration of exposure was not stated.

**DISCUSSION FROM 1997 PUBLISHED CIR FINAL REPORT**

The results of a skin irritation and sensitization test indicated that 10% m-phenylenediamine induced skin irritation (intact skin) in four of nine guinea pigs tested and that repeated applications of 1% m-phenylenediamine, followed by challenge at the same concentration, caused only mild sensitization in one animal; however, in a skin irritation test involving 10 guinea pigs (intact skin), the results were negative at both test concentrations of m-phenylenediamine (10% and 50%) in a hydrophilic ointment vehicle. Given these test results, the Expert Panel determined that m-phenylenediamine and m-phenylenediamine sulfate can be used safely in hair dyes at concentrations of up to 10%.

The Panel noted the mixed results reported in *in vitro* mutagenicity tests on m-phenylenediamine; however, the critical endpoints of carcinogenicity and teratogenicity were negative.
Table 1. Genotoxicity Studies on m-Phenylenediamine

<table>
<thead>
<tr>
<th>Mammalian Assays</th>
<th>Assay/Strains Tested</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>16, 33, or 65 mg/kg body weight; solvent: deionized water</td>
<td>Micronucleus Assay; Crl:CD™-1(ICR)BR mouse bone marrow polychromatic erythrocytes</td>
<td>Negative&lt;sup&gt;18&lt;/sup&gt;</td>
</tr>
<tr>
<td>Concentrations up to 9363 µg/ml; solvent: DMSO</td>
<td>Chromosome aberrations assay; Chinese hamster ovary cells (CHO-K1)</td>
<td>Positive dose-related increase in chromosomal aberrations.&lt;sup&gt;19&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5 to 25 mM; solvent: phosphate-buffered saline</td>
<td>Comet assay with and without (Nicotiana tabacum strain TX1) TX1MX activation mix; human lymphocytes</td>
<td>Without activation, DNA strand breaks, alkali-labile sites, and incomplete excision repair sites. With activation, dramatic reduction in genotoxicity. Though concentrations &gt; 1 mM + activation significantly increased (P ≤ 0.001) tail moment values, increase did not approach levels of DNA damage induced without activation.&lt;sup&gt;20&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µM to 5 mM; solvent: phosphate-buffered saline</td>
<td>Assay with and without plant (Nicotiana tabacum strain TX1) TX1MX activation mix; Salmonella typhimurium strain YG1024</td>
<td>Not genotoxic without activation. Significant concentration response with activation (positive response).&lt;sup&gt;20&lt;/sup&gt;</td>
</tr>
<tr>
<td>Doses up to 3000 µg/plate; solvent: DMSO</td>
<td>Assay with (S9 mix) and without metabolic activation; Salmonella typhimurium strains TA98 and TA100</td>
<td>With activation, highly genotoxic to strain TA98, but weakly genotoxic to strain TA100. Negative results without activation.&lt;sup&gt;19&lt;/sup&gt;</td>
</tr>
<tr>
<td>100 µM to 2 mM; solvent: citrate phosphate buffer</td>
<td>Plant cell/microbe coincubation assay (cultured tobacco cells [Nicotiana tabacum strain TX1] in TX1MX medium for activation); Salmonella typhimurium strains TA98, YG1024, and TA98/1,8-DNP&lt;sub&gt;e&lt;/sub&gt;</td>
<td>m-phenylenediamine + TX1MX (cell-free) more genotoxic to strains TA98 and YG1024 than m-phenylenediamine + TX1MX with tobacco cells. Genotoxicity was concentration-dependent. Neither was genotoxic to strain TA98/1,8-DNP&lt;sub&gt;e&lt;/sub&gt;.&lt;sup&gt;22&lt;/sup&gt;</td>
</tr>
<tr>
<td>Doses up to 1078 µg/plate; solvent: DMSO</td>
<td>Assay with (S9 mix) and without metabolic activation; Salmonella typhimurium strains TA98 and TA100</td>
<td>m-phenylenediamine HCl genotoxic to strain TA98, but not strain TA100.&lt;sup&gt;21&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 mM; solvent: potassium phosphate buffer</td>
<td>Assay with activation (cultured tobacco cells [Nicotiana tabacum strain TX1] in TX1MX mix or using TX1MX supernatant fraction or TX1MX gel fraction)</td>
<td>Genotoxic with activation. TX1MX gel fraction more potent in activating m-phenylenediamine into genotoxic agent than TX1MX or its supernatant fraction.&lt;sup&gt;24&lt;/sup&gt;</td>
</tr>
<tr>
<td>≤ 25 to between 250 and 300 mM; solvent: phosphate buffer</td>
<td>Assay to identify streptomycin-resistant mutants induced by m-phenylenediamine; Chlamydomonas reinhardtii strains</td>
<td>Highest rate of streptomycin-resistant mutants in strain uvs14. Not genotoxic in strain uvs12.&lt;sup&gt;26&lt;/sup&gt;</td>
</tr>
<tr>
<td>1, 2, and 4 mM; solvent: phosphate buffer</td>
<td>Assay using Saccharomyces cerevisiae strain D7</td>
<td>Frequency of revertants per 10&lt;sup&gt;9&lt;/sup&gt; survivors was elevated at highest test concentration, and was ~7- to 8-fold over the control.&lt;sup&gt;26&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5 to 60 mM; solvent: phosphate buffer</td>
<td>Algal cell/Salmonella typhimurium coincubation assay; strains TA98 and YG1024</td>
<td>Genotoxic at 30 to 60 mM in both strains.&lt;sup&gt;26&lt;/sup&gt;</td>
</tr>
<tr>
<td>1, 2, 4, or 8 mM; solvent: phosphate buffer</td>
<td>Algal supernatant/yeast assay; Saccharomyces cerevisiae strain D7</td>
<td>Frequency of revertants at highest concentration elevated 17-fold (genotoxic) when compared to control.&lt;sup&gt;26&lt;/sup&gt;</td>
</tr>
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<td>Results</td>
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<tr>
<td>2, 4, 8, 16, or 20 mM; solvent not stated</td>
<td>Cell-free activation medium/yeast assay; <em>Saccharomyces cerevisiae</em> strain D7</td>
<td>Toxic effect in this assay lower when compared to preceding assay. Therefore, higher test concentration used to achieve same toxic effect. At equitoxic concentrations, frequencies of genetic changes (genotoxicity) induced were similar to those observed in preceding assay.²⁶</td>
</tr>
<tr>
<td>50, 100, 200 or 500 µM; solvent: phosphate buffer containing CuCl₂ and DTPA</td>
<td>DNA damage assay to assess damage in presence of Cu(II);³²P-5′-end labeled DNA fragments obtained from the human c-Ha-ras-1 protooncogene and the p53 tumor suppressor gene</td>
<td>No clear DNA damage or significant autoxidation.²⁷</td>
</tr>
</tbody>
</table>
References


2012 FDA VCRP Data

m-Phenylenediamine
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests) 46

m-Phenylenediamine Sulfate
06A - Hair Dyes and Colors (all types requiring caution statements and patch tests) 19
FINAL REPORT ON THE SAFETY ASSESSMENT
OF m-PHENYLENEDIAMINE AND
m-PHENYLENEDIAMINE SULFATE

The ingredients m-Phenylendiamine and m-Phenylendiamine Sulfate are aromatic amines that function as hair colorants in cosmetic products. Both are currently used in hair dye products at concentrations of up to 3%. Percutaneous absorption of m-Phenylendiamine has been demonstrated in animals. Three metabolites excreted in urine have been identified. The oral LD<sub>50</sub> of m-Phenylendiamine in rats is between 360 and 650 mg/kg. Subchronic studies in rats (oral) indicated some lesions in the liver but no kidney injury, while one study in rabbits (dermal) indicated some liver and kidney toxicity. Another dermal study in rabbits failed to show any liver or kidney toxicity. Skin irritation and sensitization were found in guinea pigs exposed to m-Phenylendiamine. Clinical data indicated some evidence of sensitization. A short-term study in rats (oral) reported an absence of any neurotoxicity. One study in female rats identified fetotoxicity but no evidence of terata. Other studies reported neither birth defects nor fetal deaths. Both positive and negative results were found in various mutagenesis assay systems. In studies with mice and rats, neither m-Phenylendiamine (both oral and dermal exposure) nor hair dye formulations (dermal exposure only) containing m-Phenylendiamine were carcinogenic. Based on the concentrations of m-Phenylendiamine shown to produce sensitization in animal studies, it was concluded that these ingredients can be used safely in hair dyes at concentrations of up to 10%.

The available published and unpublished data relevant to assessing the safety of the hair dye ingredients m-Phenylendiamine and m-Phenylendiamine Sulfate are reviewed in this article. While almost all the available data relate to m-Phenylendiamine, the data were considered relevant also to m-Phenylendiamine Sulfate. The Cosmetic Ingredient Review (CIR) Expert Panel has reviewed the safety of a related ingredient, p-Phenylendiamine, and concluded that for individuals who are not sensitized, this ingredient is safe as a hair dye ingredient as used with the known concentrations of use ranging from ≤0.1% to 5.0% (Elder, 1985).

1Reviewed by the Cosmetic Ingredient Review Expert Panel.
This report was prepared by Wilbur Johnson, Jr., Senior Scientific Analyst and Writer.
Address correspondence to Dr. F. Alan Andersen, Cosmetic Ingredient Review, 1101 17th Street NW, Suite 310, Washington, DC 20036, USA.
CHEMISTRY

Chemical and Physical Properties

The ingredient m-Phenylenediamine (CAS No. 108-45-2) is the aromatic amine that conforms to the following formula (Wenninger and McEwen, 1995a):

\[
\text{NH}_2
\]
\[
\text{NH}_2
\]

Other names for this chemical include Metaphenylenediamine, 1,3-Phenylenediamine, m-Aminoaline, 3-Aminoaniline, m-Benzenediamine, 1,3-Benzenediamine, m-Diaminobenzene, and 1,3-Diaminobenzene (Registry of Toxic Effects of Chemical Substances [RTECS], 1994a; Wenninger and McEwen, 1995a). Technical grade m-Phenylenediamine is soluble in alcohol, ether, and water and has a minimum purity of 99% (Lewis, 1993a). Additional properties of m-Phenylenediamine are listed in Table 1.

The ingredient m-Phenylenediamine Sulfate (CAS No. 541-70-8) is the aromatic amine that conforms to the following formula (Wenninger and McEwen, 1995a):

\[
\text{NH}_2
\]
\[
\text{\cdot H}_2\text{SO}_4
\]
\[
\text{NH}_2
\]

It has a molecular weight of 206.21 (RTECS, 1994b) and is also known as 1,3-Phenylenediamine Sulfate (Wenninger and McEwen, 1995a) and 1,3-Benzenediamine Sulfate (RTECS, 1994b).

Methods of Production

The ingredient m-Phenylenediamine is produced by the reduction of metadinitrobenzene or nitroaniline with iron and hydrochloric acid. The product is then purified by crystallization (Lewis, 1993a).
Table 1. Properties of m-Phenylenediamine

<table>
<thead>
<tr>
<th>Property</th>
<th>Data</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Form</td>
<td>Rhombic crystals (in ethyl alcohol)</td>
<td>Lide and Frederiske, 1993</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>108.15</td>
<td>Lide and Frederiske, 1993</td>
</tr>
<tr>
<td>Dipole moment</td>
<td>1.79</td>
<td>Budavari et al., 1989</td>
</tr>
<tr>
<td>Boiling point</td>
<td>282–284°C</td>
<td>Lide and Frederiske, 1993</td>
</tr>
<tr>
<td>Melting point</td>
<td>63–64°C</td>
<td>Lide and Frederiske, 1993</td>
</tr>
<tr>
<td>Fire point</td>
<td>175°C</td>
<td>Budavari et al., 1989</td>
</tr>
<tr>
<td>Vapor pressure</td>
<td>1 mm @ 99.8°C</td>
<td>Sax, 1979</td>
</tr>
<tr>
<td>Density</td>
<td>1.0696 @g 55°C/4°C</td>
<td>Lide and Frederiske, 1993</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.0096 @ 58°C</td>
<td>Scientific and Technical</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Information Network International, 1994</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.6339 @ 58°C</td>
<td>Lide and Frederiske, 1993</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in ethyl alcohol, ether, benzene, and water</td>
<td>Lide and Frederiske, 1993</td>
</tr>
<tr>
<td>λmax</td>
<td>240 nm (E = 708); 293 nm (E = 263)</td>
<td>IARC, 1978</td>
</tr>
</tbody>
</table>

The ingredient m-Phenylenediamine Sulfate may be prepared by reaction of the appropriate amine with sulfuric acid (Environmental Protection Agency [EPA], 1987).

Impurities

A technical grade (99.5% pure) of m-Phenylenediamine has been available in the United States with the following specifications: water-insoluble matter (0.1% maximum), moisture content (0.1% maximum), dinitrobenzene (0.1% maximum), p-phenylenediamine (500 mg/kg maximum), o-phenylenediamine (200 mg/kg maximum), and freezing point (62.7°C minimum) (International Agency for Research on Cancer [IARC], 1978). More recent data indicate that m-Phenylenediamine contains the following contaminants: o-phenylenediamine (<200 ppm), iron (<5 ppm), p-phenylenediamine (<100 ppm), water (<0.1%), and nitro compounds (<0.1%) (Du Pont, 1992).
In Japan, m-Phenylenediamine is supplied as a technical grade, with a minimum purity of 98.5% and with a minimum melting point of 61.5°C, and it contains dinitrobenzene and phenylenediamine isomers as impurities (IARC, 1978).

Impurities that have been detected in 93% pure liquified m-Phenylenediamine include oxygen (0.3%) and sodium formate (400 ppm) (Oak Ridge National Laboratory [ORNL], 1981).

**Reactivity**

The ingredient m-Phenylenediamine (white crystalline form) becomes red after exposure to air (Budavari et al., 1989), and it is combustible when exposed to heat or flame. When heated to decomposition, toxic fumes of NOₓ are emitted (Lewis, 1993b).

In hair dyes, the oxidation of m-Phenylenediamine is rapid, and there is no build-up of diamines or imines. In aqueous media, m-Phenylenediamine undergoes rapid polymerization or hydrolysis (depending on the pH), giving rise to other products that are not formed in oxidation dyeing (i.e., quinone imines) (General Electric [GE], 1983).

**Analytical Methods**

Some of the methods that have been used in the analysis of m-Phenylenediamine include ultraviolet spectrophotometry, paper chromatography, gas chromatography, pyrolysis gas chromatography, high-performance liquid chromatography, and mass spectrometry (RTECS, 1994b).

**USE**

**Scope and Extent of Use in Cosmetics**

The ingredients m-Phenylenediamine and m-Phenylenediamine Sulfate are used as hair colorants in cosmetic products (Wenninger and McEwen, 1995b). The frequency of use data submitted to the Food and Drug Administration (FDA) in 1995 indicated that m-Phenylenediamine and m-Phenylenediamine Sulfate were used in a total of 162 and 28 cosmetic product formulations, respectively (FDA, 1995). The frequency with which, and the product categories in which, each of these ingredients is used are shown in Table 2.

Concentration of use values are no longer reported to the FDA by the cosmetics industry (FDA, 1992); however, product formulation data submitted to the FDA in 1984 indicated that m-Phenylenediamine and m-Phenylenediamine Sulfate were used at concentrations ranging from ≤0.1% to 1.0% (FDA, 1984). Data submitted to the Cosmetic, Toiletry
Table 2. Frequency of use of m-Phenylenediamine and m-Phenylenediamine sulfate

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Product category</th>
<th>Total no. of formulations in category</th>
<th>Total no. containing ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-Phenylenediamine</td>
<td>Hair dyes and colors (all types requiring caution statement and patch test)</td>
<td>1437</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td><strong>1995 Totals</strong></td>
<td></td>
<td><strong>162</strong></td>
</tr>
<tr>
<td>m-Phenylenediamine sulfate</td>
<td>Hair dyes and colors (all types requiring caution statement and patch test)</td>
<td>1437</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Hair dyes and colors (all types requiring caution statement and patch test)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>1995 Totals</strong></td>
<td></td>
<td><strong>22</strong></td>
</tr>
</tbody>
</table>

*Source. FDT, 1995.*

and Fragrance Association (CTFA) in 1995 indicated that m-Phenylenediamine and m-Phenylenediamine Sulfate were used in hair colors at concentrations of up to 3% (CTFA, 1995).

Permanent hair coloring formulations containing m-Phenylenediamine and m-Phenylenediamine Sulfate are applied to, or may come in contact with, hair, skin (particularly the scalp), eyes, and nails. Individuals dyeing their hair may use such formulations once every few weeks, whereas hairdressers may come in contact with products containing m-Phenylenediamine and m-Phenylenediamine Sulfate several times per day.

Oxidative or permanent hair dyes containing m-Phenylenediamine and m-Phenylenediamine Sulfate, 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 the label bears a caution statement and “patch test” instructions for determining whether the product causes skin irritation (FDA, 1979). In order to be exempt, the following caution statement must be displayed conspicuously on all coal tar hair dye products:

Caution—this product contains ingredients which may cause skin irritations 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.
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.

There is a general consensus among dermatologists that screening of patients for sensitization (allergic contact dermatitis) should be conducted by the procedures used by the North American Contact Dermatitis Group 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 at 48 and 72 hours after application. The CIR Expert Panel has cited the results of studies conducted by both the North American Contact Dermatitis Group and the International Contact Dermatitis Group in its safety evaluation reports on cosmetic ingredients (Elder, 1985).

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

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 cosmetics 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.

International Use

In the European Union, the maximum authorized concentration of both m-Phenylenediamine and m-Phenylenediamine Sulfate in the finished cosmetic product is 6.0\% (calculated as free base) (EEC, 1993). Neither m-Phenylenediamine nor m-Phenylenediamine Sulfate are included in the CTFA List of Japanese Cosmetic Ingredients that are known to be approved for cosmetic use in Japan. The omission of an ingredient from this list does not necessarily mean that it has never been approved for use in Japan, as new ingredients are approved on an ongoing basis by
m-PHENYLENEDIAMINE AND m-PHENYLENEDIAMINE SULFATE

the Japan Ministry of Health and Welfare. m-Phenylenediamine and m-Phenylenediamine Sulfate also are not included on the list of substances expressly prohibited from use in cosmetics manufactured or imported into Japan (Rempe and Santucci, 1997).

Noncosmetic Use

The ingredient m-Phenylenediamine is used in the process of manufacturing the following products: dyes, rubber curing agents, ion exchange resins, decolorizing resins, formaldehyde condensates, resinous polyamides, block polymers, textile fibers, urethanes, petroleum additives, rubber chemicals, and corrosion inhibitors. In photography, it is used as a reagent for gold and bromine (Budavari et al., 1989). It is also used as a curing system additive in the manufacture of 4,4'-isopropylidene-diphenolepichlorohydrin thermosetting epoxy resins. According to the Code of Federal Regulations (21 CFR 177.2280), these resins may be safely used as articles or components of articles intended for repeated use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food.

On May 28, 1980, the Interagency Testing Committee (ITC) recommended that phenylenediamines, as a class, be considered for the testing of toxic effects on human health and the environment. The ITC listed 47 chemicals, including m-Phenylenediamine and m-Phenylenediamine Sulfate, that fall within ITC's stated definition for phenylenediamines as occurring in the Toxic Substances Control Act (TSCA) Public Inventory. Because section 3 of the TSCA excludes cosmetics subject to the Food, Drug, and Cosmetic Act from TSCA jurisdiction, exposure potential as a result of hair dye use was not being considered as a basis for testing. Section 4(e) of the TSCA established an ITC to recommend to the EPA Administrator a list of chemical substances and mixtures to be considered for the development of test rules under TSCA section 4(a) (EPA, 1982).

BIOLOGIC PROPERTIES

Percutaneous Absorption and Excretion

A gel containing m-Phenylenediamine hydrochloride (1.5 g in 50 mL gel) was applied to a 500 cm² area of abdominal skin on male dogs (no. not stated; weights = 8–12 kg). At 3 h postapplication, the abdominal skin was washed several times with soap and water and thoroughly rinsed. The concentration of m-Phenylenediamine in the blood rose quickly after application, and, at 1 h, the mean concentration (five experiments) was 1.2 µg m-Phenylenediamine/mL blood. The mean
concentration (five experiments) of m-Phenylenediamine was 1.4 μg/mL blood at 3 h postapplication. There was no detectable concentration of m-Phenylenediamine in the blood 3 h after the skin was washed. The percutaneous absorption of m-Phenylenediamine was also demonstrated by monitoring the formation of ferrihemoglobin (methemoglobin) after dermal application. Increases in methemoglobin concentration were observed during the percutaneous absorption of m-Phenylenediamine in each of the five experiments. Based on the increases in methemoglobin concentration that were observed, it was inferred that approximately 60 mg m-Phenylenediamine was absorbed through the skin (Kiese et al., 1968).

The percutaneous absorption and excretion of a 4% (weight/vol) solution of 14C-m-Phenylenediamine in saline were evaluated using three groups of seven specific-pathogen-free male Wistar rats (6 weeks old; weights = approximately 200 g) by Bisgaard and Lam (1989). A 1.5-mL aliquot (556 μmol of 14C-m-Phenylenediamine) of the test solution was applied to a clipped 8 × 8-cm area on the back of each of seven animals, and urine and feces were collected over a 24-h period. The test site was covered with gauze, aluminum foil, an occlusive dressing, and an elastic dressing. The remaining two groups were exposed to water (1.5 mL) and 4% 14C-m-Phenylenediamine in 4% H2O2 (1.5 mL), respectively, according to the same procedure. All animals were killed on day 7 post-application. The principal route of excretion of 14C-m-Phenylenediamine was in the urine. The total urinary excretion of 14C-m-Phenylenediamine, expressed as percentage of in vivo absorption, was 80.7% ± 6.0%. The total fecal excretion of 14C-m-Phenylenediamine, expressed as percentage of in vivo absorption, was 10.0% ± 2.2%. For rats exposed to 14C-m-Phenylenediamine in H2O2 the excretion of 14C-m-Phenylenediamine in the feces was significantly higher. Values for radioactivity (%) bound to the skin were 0.92% ± 0.56% (n = 7) and 1.51% ± 0.44% for groups exposed to 14C-m-Phenylenediamine and 14C-m-Phenylenediamine in H2O2, respectively. In the two groups exposed to 14C-m-Phenylenediamine and 14C-m-Phenylenediamine in H2O2, three N-acetylated metabolites (N-acetyl-1,3-diaminobenzene, N,N'-diacetyl-2,4-diaminophenol, and N,N'-diacetyl-1,3-diaminobenzene) accounted for 49% and 37% of the urinary excretion, respectively (Lam and Bisgaard, 1989). In another in vivo study by the same investigators, male Wistar rats absorbed 18.0% ± 3.0% (n = 7) of the applied dermal dose of 14C-m-Phenylenediamine (dose = 240 mg/kg) during the first 24 h of exposure. The values for excretion in the urine and feces were 8.5% ± 2.2% and 0.25% ± 0.13% of absorbed m-Phenylenediamine, respectively.

Bisgaard and Lam (1989) also investigated the formation of m-Phenylenediamine metabolites in vitro to identify metabolites other
than those noted in the earlier-mentioned study. Based upon the premise that any additional metabolites would be formed mainly in the liver, the transformation of m-Phenylendiamine in three different in vitro models was studied: the perfused rat liver, microsomal rat liver preparations, and primary rat hepatocyte cultures. The results indicated that in both the perfused liver and in the primary hepatocyte cultures, $^{14}$C-m-Phenylendiamine was N-acetylated to compounds such as N,N'-diacetyl-1,3-diaminobenzene, N-acetyl-1,3-diaminobenzene, and N,N'-diacetyl-2,4-diaminophenol. Similar results were noted in in vivo studies summarized earlier; however, none of the metabolites was formed by the reconstituted liver microsomal system. This is an expected result in that the hepatic N-acetyltransferases are located mainly in the cytosol of intact hepatocytes and because microsomal preparations are depleted of the coenzyme AcCoA, which is utilized in the acetyltransferase-catalyzed reaction.

These authors also evaluated the in vitro percutaneous absorption of $^{14}$C-m-Phenylendiamine using excised skin from 6-week-old male Wistar rats. Epidermal membranes were clamped between the two halves of two-chambered glass diffusion cells and pressed against a stainless steel supporting screen by a hydrostatic pressure of approximately 5 mm water. The area of epidermis exposed was 1.8 cm$^2$. During the 48-h incubation period (temperature = 30 ± 1°C), a 4% (weight/vol) solution of $^{14}$C-m-Phenylendiamine and tritiated water in either 0.9% (weight/vol) saline or 4% (weight/vol) hydrogen peroxide in 0.9% saline was applied to the donor half of the cell and 3000 μL 0.9% saline was applied to the receptor half. The calculated in vitro flux was 0.77 μmol/cm$^2$/h. The authors stated that the in vitro flux across the isolated rat epidermis is in remarkable agreement with the flux across the entire occluded skin in vivo. This suggests that permeability studies in vitro using isolated epidermal membranes from rats may be used to predict the percutaneous absorption, by rats in vivo, of m-Phenylendiamine and compounds with similar physical and chemical properties (Lam and Bisgaard, 1989).

In rabbits that received oral doses of radiolabeled 1,3-dinitrobenzene (dose = 50–100 mg/kg), m-Phenylendiamine (1,3-diaminobenzene) was a major urinary metabolite. The excretion of metabolites in the urine and feces was monitored for 2 days. The metabolism of 1,3-diaminobenzene to 2,4-diaminophenol, another urinary metabolite, was also noted (Rickert, 1987).

**Other Biologic Effects**

Two groups of five Fischer female rats (6 weeks old) received concentrations of 1000 ppm and 2000 ppm m-Phenylendiamine, respectively, in
the diet (Wayne Blox meal) daily for 6 weeks. A control group of five rats received meal only. m-Phenylenediamine was not goitrogenic and did not have any other effect on the morphology of the thyroid gland; however, mitotic figures that are rarely observed in the thyroid gland were observed in two animals. In the same study, m-Phenylenediamine prevented the dark pigmentation of the thyroid epithelium that was induced by 2,4-diaminoanisole in Fischer female rats; both substances were fed in the diet (Evarts and Brown, 1981).

The effect of m-Phenylenediamine Hydrochloride on the circulatory system was evaluated using an 11-kg dog that had been curarized. After intravenous injection of the test substance (dose = 0.0062 g/kg), the following observations were made: moderate rise in blood pressure, initial slowing of pulse, and cardiac dilatation followed by increased pulse rate and systolic tendency of the heart. There was very little change in kidney volume (Hanzlik, 1922).

Two guinea pigs (weights not stated) were injected intravenously with 0.11 and 0.3 g/kg doses of m-Phenylenediamine Hydrochloride, respectively. Both animals had anaphylactoid symptoms, which consisted of moderate dyspnea and increased respiratory rate; these effects did not cause death. At necropsy, the lungs of both animals were distended and hemorrhagic (Hanzlik, 1922).

Tainter et al. (1929) studied the effect of subcutaneous injection of m-Phenylenediamine in rabbits in three animal strains. In the first, the induction of edema after the subcutaneous injection of m-Phenylenediamine at applied doses ranging from 0.15 g to 0.45 g was evaluated using eight rabbits (weights not stated). The results were as follows: One rabbit dosed with 0.15 g m-Phenylenediamine had no signs. Signs were observed in two of four rabbits dosed with 0.2 g m-Phenylenediamine. One rabbit had a thoracic effusion of 13.5 mL and a trace of free fluid in the abdomen; the other rabbit had 40 mL of exudate in the abdomen and none in the thorax. There was no difference in the severity of signs observed in three rabbits dosed with 0.3 g and 0.45 g m-Phenylenediamine versus those observed in rabbits dosed with 0.2 g. At necropsy of the eight rabbits, no evidence of edema was found in abdominal organs or muscle and skin from various regions of the tongue, larynx, etc. In the second experiment, m-Phenylenediamine was injected subcutaneously into 11 rats (weights not stated) at doses ranging from 0.08 g to 2.0 g. No signs were noted at the highest dose administered. Necropsy results indicated no abnormal collections of fluid or edema. In a third experiment, m-Phenylenediamine was subcutaneously injected into seven guinea pigs (weights not stated) at doses of up to 1 g. No effects were noted within 24 h of administration of the highest dose. At a dose of 0.15 g, exudation of fluid into the pleural and
peritoneal cavities was noted in five guinea pigs. The two largest collections of fluid (19.5 mL and 11 mL) were found in the thorax. The largest collection of fluid in the abdomen was only 4 mL. Necropsy results for all animals did not indicate changes in any other tissues.

TOXICOLOGY

Acute Inhalation Toxicity

The acute inhalation toxicity of m-Phenylenediamine distilled flakes was evaluated using ten Sprague-Dawley rats (both sexes; weights not stated). The animals were placed in a chamber and exposed to the test substance (dust particles) for 6 h. Particle size ranged from 1.75 to 17.5 μm; 80% of the particles had a size of less than 5 μm. Exposure was followed by a 14-day observation period. All animals survived the 6-h exposure period; however, nine animals died within 24 to 48 h after removal from the chamber. Necropsy results for animals that died indicated pronounced pulmonary congestion with occasional hemorrhages. The results of a necropsy performed on the surviving animal also indicated marked pulmonary congestion (Scientific Associates, 1961).

In another study, groups of 10 rats (7–8 wks old; 236–278 g) were exposed (single 4-h exposures; nose only) to atmospheres containing m-Phenylenediamine. During the exposure period, red ocular and nasal discharge was noted at all concentrations that were tested. Mean test concentrations ranged from 0.72 mg/L to 3.9 mg/L. The following findings were reported during the 14-day observation period. Hair loss and moderate to severe weight loss, followed by weight gain, were noted at all concentrations tested. At concentrations of greater than 2.0 mg/L, lung noise, brown stained fur, pallor, and tremors were noted. Labored breathing, red ocular and nasal discharge, and lethargy were observed at concentrations of greater than 3.2 mg/L. An LC₅₀ of 3.2 mg/L (95% confidence limits of 2.6 and 4.1 mg/L) was calculated (Du Pont, 1982).

Acute Oral Toxicity

The results of six acute oral toxicity studies are summarized in Table 3.

In the first study, two pairs of male albino rats (Spartan strain; weights = 235–250 g) received oral doses of 500 and 5000 mg/kg m-Phenylenediamine in corn oil, respectively. Each dose was administered at a volume of 10 mL/kg. Both animals dosed with 5000 mg/kg m-Phenylenediamine died; the 500 mg/kg dose did not cause death. m-Phenylenediamine was classified as a toxic material (International Research and Development Corporation [IRDC], 1974).
<table>
<thead>
<tr>
<th>Test substance</th>
<th>Number of animals</th>
<th>Test procedure</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-Phenylenediamine (in corn oil)</td>
<td>Four albino rats (235–250 g)</td>
<td>Single oral doses (two rats given 500 mg/kg and two rats given 5000 mg/kg)</td>
<td>No deaths at 500 mg/kg; 100% mortality at higher dose</td>
<td>IRDC, 1974</td>
</tr>
<tr>
<td>m-Phenylenediamine (in oil or in water emulsion)</td>
<td>10 Charles River CD rats (200–300 g)</td>
<td>Single oral dose</td>
<td>LD_{so} = 650 mg/kg</td>
<td>Burnett et al., 1977</td>
</tr>
<tr>
<td>m-Phenylenediamine (suspension in peanut oil)</td>
<td>Eight CbR-CD rats (weights not given)</td>
<td>Doses = 200 to 3400 mg/kg; different dose per rat</td>
<td>Four rats receiving 200–3400 mg/kg died; 200–670 mg/kg—no deaths; 1000 mg/kg = approximate lethal dose</td>
<td>Du Pont, 1966a</td>
</tr>
<tr>
<td>m-Phenylenediamine (25% suspension in peanut oil)</td>
<td>Albino rats (number and weights not given)</td>
<td>Single doses by stomach tube</td>
<td>Lethal doses = 450–2250 mg/kg; sublethal doses = 130–300 mg/kg</td>
<td>Du Pont, 1956</td>
</tr>
<tr>
<td>m-Phenylenediamine distilled flakes (20% aqueous solution)</td>
<td>Six groups of five Sprague-Dawley albino rats (weights not given)</td>
<td>Single doses by rubber catheter</td>
<td>LD_{so} = 360 mg/kg</td>
<td>Scientific Associates, 1961</td>
</tr>
<tr>
<td>m-Phenylenediamine distilled flakes (20% aqueous solution)</td>
<td>Six New Zealand albino rabbits (weights not given)</td>
<td>Single oral dose</td>
<td>Minimal lethal doses = 250–500 mg/kg</td>
<td>Scientific Associates, 1961</td>
</tr>
</tbody>
</table>
m-PHENYLENEDIAMINE AND m-PHENYLENEDIAMINE SULFATE

In the second report, the acute oral LD$_{50}$ for m-Phenylenediamine, in oil-in-water emulsion, in 10 Charles River CD rats (5 males, 5 females; weights = 200–300 g) was 650 mg/kg (Burnett et al., 1977).

In the third study, single doses of m-Phenylenediamine (suspension in peanut oil) were administered by intragastric intubation to eight CbR-CD male rats (weights not stated). Each animal received a different dose, and the doses administered ranged from 200 mg/kg to 3400 mg/kg. Animals that survived were killed at day 14 postadministration. Administration of the four doses ranging from 1000 mg/kg to 3400 mg/kg resulted in death. None of the four rats dosed with 200 mg/kg to 670 mg/kg died. Pathologic changes observed in animals that died included pulmonary edema, hemorrhage and congestion, and focal necrosis in splenic follicles at a dose of 1500 mg/kg. There were no test substance-related pathologic changes in animals that survived. m-Phenylenediamine was classified as moderately toxic, and the approximate lethal dose was 1000 mg/kg (Du Pont, 1966a).

In the fourth, the acute oral toxicity of m-Phenylenediamine was evaluated using male albino rats (number and weights not stated). The test substance was administered by stomach tube as a 25% suspension in peanut oil. Reduced activity and evidence of discomfort were noted within 15 min postdosing in rats that received lethal doses in the 450-mg/kg to 2250-mg/kg dose range. Increased urine (color = deep yellow) volume and dyspnea were observed within 1 h, and death usually occurred within 24 h. Transient discomfort, reduced activity, and failure to gain weight normally during the first week of dosing were observed in rats that received sublethal doses in the 130-mg/kg to 300-mg/kg dose range. These rats also voided urine that was described as abnormally yellow. At necropsy and microscopic examination of tissues, the following changes were observed in rats that received lethal doses: pleural effusion, pulmonary congestion and edema, hemorrhagic areas in the squamous portion of the stomach, and marked disintegration of the kidney tubular epithelium. For rats that received sublethal doses down to 130 mg/kg, no lesions other than foci of nephrosis were noted after the animals were killed on day 9 or 10 postdosing. Pathologic changes were not observed at doses below 130 mg/kg (Du Pont, 1956).

In the fifth study, the acute oral toxicity of m-Phenylenediamine distilled flakes was evaluated using six groups of five Sprague-Dawley albino rats (male and female). The six groups received a 20% aqueous solution of the test substance in doses of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.8 g/kg, respectively. Each dose was administered using a hypodermic syringe attached to a rubber catheter. The oral LD$_{50}$ was 0.36 g/kg, and the majority of the animals died within 36 h postadministration. Minor to severe clonic convulsions were observed prior to death. At necropsy,
### Table 4. Acute dermal toxicity of m-Phenylenediamine

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Number of animals</th>
<th>Test procedure</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-Phenylenediamine (undiluted)</td>
<td>Four New Zealand white rabbits (2352–2493 g)</td>
<td>Doses of 200 and 2000 mg/kg applied for 24 h to two pairs of rabbits, respectively</td>
<td>No deaths</td>
<td>IRDC, 1974</td>
</tr>
<tr>
<td>m-Phenylenediamine distilled flakes (in thick paste made with corn oil)</td>
<td>Six New Zealand white rabbits (weights not stated)</td>
<td>Single application at doses of 0.25–5.0 g/kg</td>
<td>The three rabbits dosed with 1, 2, and 5 g/kg died. Minimum lethal doses was in the range of 0.75–1.0 g/kg</td>
<td>Scientific Associates, 1961</td>
</tr>
<tr>
<td>m-Phenylenediamine (in oxidation-type hair dye base)</td>
<td>Four to eight New Zealand rabbits (weights = 1.7–2.5 kg)</td>
<td>Single 24-h application</td>
<td>Deaths (number not stated) observed at 5-g/kg dose</td>
<td>Burnett et al., 1977</td>
</tr>
<tr>
<td>m-Phenylenediamine (15% suspension in polypropylene glycol and 50% in hydrophilic ointment)</td>
<td>Three albino rabbits (weights = 2.5–2.8 kg)</td>
<td>Single application of 15% suspension (dose = 28 mL, one rabbit). Hydrophilic ointment applied to two rabbits at total doses of 37 and 56 g, respectively</td>
<td>No clinical signs of toxicity at any time during the study; neither gross nor microscopic changes related to test substance administration were noted</td>
<td>Du Pont, 1966b</td>
</tr>
<tr>
<td>40% solution of m-Phenylenediamine in 50% aqueous ethanol</td>
<td>Three rabbits (strain and weights not stated)</td>
<td>Single doses of 1000, 1500, and 2250 mg/kg, respectively</td>
<td>One animal died (1500 mg/kg dose) within 24 h</td>
<td>Du Pont, 1956</td>
</tr>
</tbody>
</table>
gastrointestinal inflammation with occasional hemorrhages and the accumulation of pleural fluids were noted (Scientific Associates, 1961).

In the sixth study, the acute oral toxicity of m-Phenylenediamine distilled flakes was evaluated using six New Zealand albino rabbits (weights not stated). Each animal received a single oral dose of the test substance (20% aqueous solution); doses ranged from 0.05 g/kg to 2.0 g/kg. The minimal lethal dose was in the range of 0.25 g/kg to 0.5 g/kg; deaths occurred within 18 h postadministration. At necropsy of animals that died, inflammation of the gastrointestinal tract and discoloration of the liver were observed (Scientific Associates, 1961).

**Acute Dermal Toxicity**

The results of five acute dermal toxicity studies are summarized in Table 4.

In the first, the acute dermal toxicity of m-Phenylenediamine was evaluated using four New Zealand white rabbits (weight range = 2352–2493 g). The test substance was applied to shaved sites on the back of each animal; doses of 200 mg/kg and 2000 mg/kg m-Phenylenediamine were administered to two pairs of rabbits, respectively. Each test site was wrapped with a gauze bandage and plastic wrap for 24 h, after which bandages were removed and sites washed with tepid tap water. None of the rabbits died during the 14-day observation period, and m-Phenylenediamine was classified as nontoxic (IRDC, 1974).

In the second study, the acute dermal toxicity of m-Phenylenediamine distilled flakes was evaluated using six New Zealand white rabbits (weights not stated). A single application of the test substance (in thick paste made with corn oil) was made to clipped skin of the back of each animal; the doses applied ranged from 0.25 g/kg to 5.0 g/kg. Each test site was covered with a plastic shield such that the test substance was maintained in close contact with the skin. Application of the test substance was followed by a 14-day nontreatment period. The three animals dosed with 1.0, 2.0, and 5.0 g/kg, respectively, died within 24 h. At necropsy, inflammation of underlying tissues at the application site was observed. Single doses of 0.25, 0.50, and 0.75 g/kg did not result in death of the remaining three animals, respectively. The minimum lethal dose was in the range of 0.75 g/kg to 1.0 g/kg (Scientific Associates, 1961).

In the third report, the dermal toxicity of m-Phenylenediamine was evaluated using four to eight New Zealand rabbits (weights = 1.7 to 2.5 kg). The test substance (in 10 mL of an oxidation-type hair dye base) was applied to shaved skin for 24 h. Deaths (number not stated) were observed at a dose of 5 g/kg; the LD$_{50}$ was not reported (Burnett et al., 1977).
In the fourth study, m-Phenylendiamine (15% suspension in polypropylene glycol; total dose = 28 mL) was applied to the skin of one male albino rabbit through a single layer of gauze that had been wrapped around the trunk. The test substance (as a paste) in hydrophilic ointment was applied at a concentration of 50% to two rabbits. The two rabbits received total doses of 37 g and 56 g, respectively. Animal weights ranged from 2.5 kg to 2.8 kg. After test substance application, the three rabbits were each wrapped with impervious film, gauze, and an elastic bandage. At 24 h post-application, the wrappings were removed and application sites washed with water and dried. After a 14-day observation period, necropsy was performed on all three rabbits. Major tissues from the rabbit that received a total dose of 56 g m-Phenylendiamine (50%) were examined microscopically. No clinical signs of toxicity were observed, nor were there any gross or microscopic changes that were related to test substance administration (Du Pont, 1966b).

In the fifth study, a 40% solution of m-Phenylendiamine in 50% aqueous ethanol was applied to the shaved skin of three rabbits (strain and weights not stated) at doses of 1000, 1500, and 2250 mg/kg, respectively. Test sites were covered (24 h) with moisture-proof cellophane and gauze. The rabbit receiving the 1500-mg/kg dose died within 24 h; pleural effusion and massive pulmonary edema were observed at necropsy. Pulmonary congestion, but not pleural effusion or massive pulmonary edema, was observed in the rabbit dosed with 2250 mg/kg. Pathologic changes were not observed after application of the 1000-mg/kg dose (Du Pont, 1956).

**Acute Subcutaneous Toxicity**

In a study involving DD mice (number and weights not stated), the LD$_{50}$ was 90 mg/kg (Saruta et al., 1962).

**Acute Intraperitoneal Toxicity**

The acute intraperitoneal LD$_{50}$ for m-Phenylendiamine (in 10% aqueous dimethylsulfoxide) was 283 mg/kg in a study involving 10 male Charles River CD rats (weights = 200–300 g) (Burnett et al., 1977).

**Short-Term Oral Toxicity**

The short-term oral toxicity of m-Phenylendiamine was evaluated using six rats (strain and weights not stated). The test substance (3% suspension in peanut oil; 90 mg/kg dose) was administered by stomach tube 5 days per week for a total of 10 doses. Three animals were killed
m-PHENYLENEDIAMINE AND m-PHENYLENEDIAMINE SULFATE

after the tenth treatment, and the remaining three, 10 days later. During the treatment period, both a slight decrease in activity and slight depression of growth were noted. Minimal nephrosis was observed in one of the three rats that was killed after the tenth treatment. Pathologic changes were not observed in the remaining five animals (Du Pont, 1956).

In another study, a test material consisting of 42% m-Phenylendiamine and 58% cumene diamine was administered (intragastric intubation; 134 mg/kg) as a 2% solution in acetone:corn oil (15:85) to each of six young adult Chr-CD male rats five times per week for 2 weeks. The effective test concentration of m-Phenylendiamine was 0.8%. Animal weights were not stated. The vehicle control group (six rats) was dosed with acetone:corn oil (15:85) according to the same procedure. Three rats per group were killed at approximately 4 h post-administration of the last dose, and the remaining rats were killed after 14 days. There were no test substance-related pathologic changes in any of the tissues that were examined microscopically. It was concluded that there was no evidence of cumulative toxicity (Du Pont, 1973a).

Short-Term Dermal Toxicity

Groups of five 10- to 12-week-old male and female C3HF/Bd and C57BL/6Bd mice received daily applications of m-Phenylendiamine in acetone (50 μL; concentrations of 10% and greater) 5 days per week for 2 weeks. Death or morbidity was noted in both strains (number of deaths not stated). At necropsy of the mice, pale and swollen livers and kidneys, indicative of hepatorenal toxicity, were observed (Holland et al., 1979).

Subchronic Oral Toxicity

From 5.6 to 28 mg/kg/day of m-Phenylendiamine was administered orally to rats (number, strain, and weights not stated) for 3 to 4 months. Urinary excretion of hippuric acid was decreased, and dermatitis was observed following the administration of 28 mg/kg. The specific effects on the central nervous system and on the detoxifying capacity of the liver that resulted were not stated (GE, 1983).

The results of a 4-week dose range-finding study in rats conducted at the Seibersdorf Research Center (SRC, 1982) prior to a subchronic oral toxicity study were as follows: Increased hemoglobin content, retarded body weight development, and a behavioral change (sedative effect) were observed in the highest-dose group (100 mg/kg/day). A behavioral change (sedative effect) was also noted in the intermediate dose group.
(30 mg/kg/day). No differences were noted between the lowest-dose group and the control group. In actual study, aqueous solutions of m-Phenylenediamine, 99% pure, were administered (by esophageal tube) to three groups of 20 male and 20 female OFA(SD)SPD rats at doses of 2.0, 6.0, and 18.0 mg/kg/day (dose volume = 10 mL/kg), respectively, for 90 days. The negative control group (20 males, 20 females) was dosed with deionized water. At the beginning of the study, mean body weights for male and female rats were 157 g and 136 g, respectively. There were no test substance–related deaths during the study. A dose-dependent, significant increase in absolute and relative liver weights was noted in the 18.0-mg/kg dose group. At histopathologic evaluation, degenerative lesions in the liver, with a significant increase in the frequency of nuclear pyknosis, were observed only in the 18.0-mg/kg dose group (males and females). A significant increase in kidney weight was observed only in female rats dosed with 18.0 mg/kg m-Phenylenediamine; however, no indications of toxic injury of any kind were observed in the kidneys. Test substance-related changes in the following parameters were not observed: body weight development, feed consumption, water consumption, ophthalmoscopy, hematology, biochemistry of the blood, and urinalysis. No conclusions concerning a test substance–related effect could be made based on necropsy findings in animals that survived to the end of the study. Necropsy results included isolated evidence of various spontaneous alterations, such as congenital fissurations of the spleen and liver nodules or testicular atrophy. Toxic effects were not observed in rats dosed with 2.0 or 6.0 mg/kg m-Phenylenediamine. It was concluded that the highest daily dose that could be administered to a group without cumulative damage and/or substance-related changes (no-effect-level) was 6 mg m-Phenylenediamine/kg body weight.

Subchronic Dermal Toxicity

The subchronic dermal toxicity of an oxidative dye formulation containing 1.5% m-Phenylenediamine was evaluated using 12 adult New Zealand white rabbits (six males, six females; weights not stated). Three groups of 12 untreated rabbits served as controls. The dye was mixed with an equal volume of 6% hydrogen peroxide and applied (dose = 1 mL/kg) to the dorsolateral aspects of the thoracic-lumbar area (one on each side of the midline). The test sites were alternated in order to minimize skin irritation. All application sites were clipped free of hair, and those on three male and three female rabbits were abraded. After each application of the dye, the animals were restrained in holding stocks for 1 h, and sites were shampooed, rinsed, and dried. The animals were killed after the thirteenth week and examined for gross
abnormalities. The only significant hematologic observation between experimental and control groups was a statistically significant increase in methemoglobin; this observation was not considered to be of toxicologic significance. The authors stated that there was no evidence of test substance-induced toxicity (Burnett et al., 1976).

Ocular Irritation

The ocular irritation potential of m-Phenylendiamine distilled flakes was evaluated using three albino rabbits. The test substance (20% aqueous solution; 0.1 mL) was instilled into the conjunctival sac of the right eye of each animal. Ocular irritation reactions were scored up to 1 week postinstillation according to the Draize scale: 0 to 110 (Draize et al., 1944). The following reactions were observed through 48 h postinstillation: erythema of the palpebral membrane, chemosis, lacrimation, and diffuse opacity of the entire cornea. The average Draize scores at 1 h and 72 h were 26.0 and 19.2, respectively. Reactions were not observed at 1 week postinstillation. After fluorescein staining of the cornea, permanent damage was not observed (Scientific Associates, 1961).

In a different study, an aliquot of m-Phenylendiamine was ground, using a mortar and pestle, and placed (10 mg) into each conjunctival sac of an albino rabbit. An equivalent amount of coarse particles mixed with mineral oil was placed in both eyes of a second rabbit. At 20 sec postinstillation, the left eyes were rinsed with tap water; the right eyes were not rinsed. Using a hand slit lamp, the cornea, iris, and conjunctiva were observed at 1 h and 3 h and at days 1, 2, 3, and 7. For examinations on the day after treatment, a biomicroscope was used after staining with 5% fluorescein. Mild, transient conjunctival irritation, without observed corneal or iridic effect, was observed in rinsed and unrinsed eyes of both rabbits (Du Pont, 1966b).

The ocular irritation potential of m-Phenylendiamine was evaluated using two rabbits (one male, one female). The test substance (100 mg) was instilled into the conjunctival sac of one eye per animal. Reactions were scored at 24, 48, and 72 h postinstillation according to the Draize scale: 0 to 110. Total ocular irritation scores of 32.5 (at 24 h), 35.3 (at 48 h), and 36.8 (at 72 h) were reported. m-Phenylendiamine was classified as an ocular irritant (IRDC, 1974).

In another study, the ocular irritation potential of m-Phenylendiamine was evaluated using two male albino rabbits. The test substance (10 mg) was instilled into the right conjunctival sac of each animal. The treated eye of only one rabbit was rinsed after instillation. The cornea, iris, and conjunctiva were observed using an ophthalmoscope at 1 h and 4 h and at 1, 2, 3, 7, and 14 days. A biomicro-
scope was used for evaluations after the day of treatment. In rinsed and unrisned eyes, m-Phenylenediamine induced the following reactions: small area of mild corneal cloudiness, moderate iritis, and moderate conjunctivitis with copious discharge. Vascularization in the lower part of the cornea was observed in the rinsed eye at day 7. Rinsed and unrisned eyes were normal within 14 days postinstillation. It was concluded that m-Phenylenediamine was a moderate ocular irritant (Du Pont, 1981).

**Skin Irritation**

The results of five skin irritation studies are summarized in Table 5. In the first study, the primary skin irritation potential of m-Phenylenediamine was evaluated using two New Zealand white rabbits (one male, one female); the male and female rabbits weighed 2344 g and 2521 g, respectively. The test substance (500 mg) was applied to shaved skin (male rabbit) and shaved and abraded skin (female rabbit) of the back, and the sites were wrapped with a gauze bandage and plastic wrap. At 24 h postapplication, the occlusive covering was removed and sites were washed with tepid water. Reactions were scored at 24 h and 72 h postapplication according to the following scales: 0 (no erythema) to 4 (severe erythema to slight eschar formation) and 0 (no edema) to 4 (severe edema, raised more than 1.0 mm and extending beyond the area of exposure). m-Phenylenediamine was not a primary skin irritant (Primary Irritation Score = 0.5) (IRDC, 1974).

In the second study, the skin corrosion potential of m-Phenylenediamine, as supplied, was evaluated using six albino rabbits (weights = 2–3 kg). The back of each animal was clipped free of hair and the test substance (0.5 g) was applied under a gauze pad. The trunk was then loosely wrapped with rubber sheeting. At 4 h postapplication, reactions were evaluated and sites washed. Reactions were also evaluated at 24 h and 48 h postapplication. m-Phenylenediamine was classified as a corrosive material (Du Pont, 1973b).

In the third report, the skin irritation potential of m-Phenylenediamine distilled flakes was evaluated using three New Zealand white rabbits. The test substance (in paste made with corn oil) was applied to abraded and intact skin sites, clipped free of hair, on the back of each animal. Each site was covered with a plastic shield for 24 h. Sites were scored at 48 h and 72 h postapplication. Minimal erythema and edema were observed within 24 h postapplication; reactions were not observed at 48 h. At 24 h, average skin irritation scores of 0.6 and 1.3 (maximum score attainable = 8) were reported for intact and abraded sites, respectively (Scientific Associates, 1961).
Table 5. Skin irritation potential of m-Phenylenediamine

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Number of animals</th>
<th>Test procedure</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-Phenylenediamine (undiluted)</td>
<td>Two New Zealand white rabbits</td>
<td>500 mg applied, under occlusive covering for 24 h, to shaved and abraded skin</td>
<td>Not a primary skin irritant</td>
<td>IRDC, 1974</td>
</tr>
<tr>
<td>m-Phenylenediamine (as supplied)</td>
<td>Six albino rabbits</td>
<td>0.5 g applied, under occlusive covering for 4 h, to skin clipped free of hair</td>
<td>Classified as corrosive material</td>
<td>Du Pont, 1973b</td>
</tr>
<tr>
<td>m-Phenylenediamine distilled flakes (in paste made with corn oil)</td>
<td>Three New Zealand white rabbits</td>
<td>Applied, under plastic shield for 24 h, to abraded and intact skin clipped free of hair</td>
<td>Transient minimal erythema and edema</td>
<td>Scientific Associates, 1961</td>
</tr>
<tr>
<td>42% m-Phenylenediamine/58% cumene diamine mixture</td>
<td>Six Albino rabbits</td>
<td>0.5 mL applied, under occlusive covering for 4 h, to skin clipped free of hair</td>
<td>Not a corrosive material</td>
<td>Du Pont, 1973c</td>
</tr>
<tr>
<td>m-Phenylenediamine (in hydrophilic ointment)</td>
<td>10 guinea pigs</td>
<td>10% and 50% test concentrations (0.06–0.08 g) applied to abraded and intact skin</td>
<td>Mile erythema at abraded sites; no erythema at intact sites</td>
<td>Du Pont, 1966b</td>
</tr>
</tbody>
</table>
In the fourth study, the skin corrosion potential of a test material consisting of 42% m-Phenylenediamine and 58% cumene diamine was evaluated using six albino rabbits (weights = 2–3 kg). The test substance (0.5 mL) was applied to dorsal skin that had been clipped free of hair. The test site on each rabbit was covered with a cotton gauze pad, and the trunk loosely wrapped with rubber sheeting. Patches were removed at 4 h postapplication, after which sites were washed. Test sites were reevaluated at 24 h and 48 h postapplication. Skin corrosion was not observed in any of the animals tested (Du Pont, 1973c).

In the last study, the skin irritation potential of m-Phenylenediamine (as a paste) in hydrophilic ointment was evaluated at concentrations of 10% and 50% using 10 male albino guinea pigs. The test substance (approximately 0.06–0.08 g) was applied to abraded and intact skin sites. Reactions were scored at days 1 and 2 postapplication according to the following scale: 1+ (mild erythema) to 4+ (necrosis). There were no skin irritation reactions to 10% or 50% m-Phenylenediamine at intact skin sites during either of the two grading periods. At abraded sites, ten 1+ reactions to 50% m-Phenylenediamine and one negative and nine 1+ reactions to 10% m-Phenylenediamine were observed at day 1. With the exception of one 1+ reaction (abraded skin), there were no skin irritation reactions at day 2 (Du Pont, 1966b).

**Skin Sensitization**

The results of four skin sensitization studies are summarized in Table 6.

In the first study, mild erythema was noted following the application of a 35% aqueous solution of m-Phenylenediamine to intact skin of male albino guinea pigs (number and weights not stated). Similar results were reported after 25% aqueous m-Phenylenediamine was applied to lightly abraded skin. The animals were retested after receiving a total of nine sensitizing applications of m-Phenylenediamine. The ninth application was followed by a 2-week nontreatment period. Intense erythematous reactions, indicative of skin sensitization, were observed (Du Pont, 1956).

In the second report, the skin irritation and sensitization potential of m-Phenylenediamine was evaluated using nine Hartley albino guinea pigs. In the skin irritation test, m-Phenylenediamine was applied to the flanks of each animal at concentrations ranging from 0.1% to 10.0%; sites were covered with occlusive patches for 48 h. m-Phenylenediamine (10%) induced skin irritation in four of the nine animals; however, skin irritation was not observed at concentrations of 0.1% to 5.0%. During the induction phase of the sensitization test, 1% m-Phenylenediamine (in approximately 50 mg of white petrolatum)
### Table 6. Skin irritation and sensitization potential of m-Phenylendiamine

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Number of animals</th>
<th>Test procedure</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-Phenylendiamine (35% and 25% aqueous)</td>
<td>Guinea pigs (number not stated)</td>
<td>Single applications of 35% and 25% aqueous to intact and abraded skin, respectively; retest after nine sensitizing applications of m-Phenylendiamine</td>
<td>Mild erythema following single applications to abraded and intact skin; intense erythematous reactions, indicative of sensitization, after repeated applications</td>
<td>Du Pont, 1956</td>
</tr>
<tr>
<td>m-Phenylendiamine (0.1–10.0%)</td>
<td>Nine Hartley albino guinea pigs</td>
<td>48-h occlusive patch test at concentrations of 0.1% to 10.0%; in second test, six 48-h induction applications (1%) followed by challenge with 0.1% and 1.0%</td>
<td>Skin irritation only with 10% m-Phenylendiamine; mild sensitization at 1.0% and no sensitization at 0.1%</td>
<td>Ishihara et al., 1985</td>
</tr>
<tr>
<td>m-Phenylendiamine (undiluted)</td>
<td>Guinea pigs (number not stated)</td>
<td>Eight oral doses at 3- to 4-day intervals</td>
<td>Allergic responses observed; antibodies detected in blood</td>
<td>GE, 1983</td>
</tr>
<tr>
<td>m-Phenylendiamine (1%)</td>
<td>Four JY-1 guinea pigs</td>
<td>Sensitized with p-Phenylendiamine, according to maximization test procedure, then challenged with 1% m-Phenylendiamine</td>
<td>No positive challenge reactions; m-Phenylendiamine did not cross-react with p-Phenylendiamine.</td>
<td>Shigematsu et al., 1988</td>
</tr>
</tbody>
</table>
was applied to the nape of each animal, and the sites were covered with occlusive patches for 48 h; applications were made three times per week for 2 weeks. After a 2-week nontreatment period, challenge concentrations of m-Phenylenediamine (0.1% and 1%) were applied to the flank of each animal, and the application sites were covered with occlusive patches for 48 h. Reactions were scored 24 h and 48 h after patch removal. There were no sensitization reactions to 0.1% m-Phenylenediamine; however, m-Phenylenediamine (1%) induced mild sensitization (sensitization rate = 10%) (Ishihara et al., 1985).

In the third study, allergic responses were observed in guinea pigs (number and weights not stated) that received eight oral doses of m-Phenylenediamine (0.001 mg) at 3- to 4-day intervals. Antibodies to m-Phenylenediamine were detected in the blood (GE, 1983).

In the final sensitization study, the cross-sensitization potential of m-Phenylenediamine was evaluated using male and female inbred strain JY-1 guinea pigs (weights = 300–400 g). Four guinea pigs were sensitized with p-Phenylenediamine according to the maximization test procedure (Magnusson and Kligman, 1969) and later challenged with 1.0% m-Phenylenediamine. Challenge applications were made to the flank, and reactions were scored at 24, 48, and 72 h according to the following scales: 0 (no erythema) to 4 (severe erythema to slight eschar formation); 0 (no edema) to 4 (severe edema: raised more than 1 mm and extending beyond the area of exposure). The results indicated that m-Phenylenediamine did not cross-react with p-Phenylenediamine. There were no positive challenge reactions (Shigematsu et al., 1988).

### Neurotoxicity

The neurotoxicity of m-Phenylenediamine (98% pure) was evaluated using eight groups of ten Crl:CDBR rats (21 days old). Half of the groups consisted of male rats. Two of the groups (10 males, 10 females) served as negative controls. Mean body weights for male and female rats were 63.7 ± 7.8 g and 55.2 ± 5.1 g, respectively. The three groups of experimental rats per sex (six groups) were dosed with 5, 10, and 20 mg/kg m-Phenylenediamine in sterile water (dosage volume = 10 mL/kg), respectively, once daily for a minimum of 90 consecutive days. Negative control groups were dosed with sterile water according to the same procedure. During the week prior to the onset of dosing and the fourth, eighth, and thirteenth weeks of dosing, a neurotoxicity test battery was conducted. The test battery consisted of motor activity and functional observational battery assessments. At the end of dosing, all surviving animals were killed and perfused, and neuropathologic examinations were performed. There were no test substance–related
deaths. During the course of the entire 13-week dosing period, a small reduction (not significant) in average body weight gain was observed only in female rats of the 20-mg/kg dose group. These changes in body weight were not test substance–related. Compared to the control group, small, dose-dependent reductions in horizontal motor activity counts (not significant) were noted in male rats from 10 mg/kg and 20 mg/kg dose groups. Concomitant reductions in vertical activity counts (not significant) were also observed in male rats that received 20 mg/kg doses. The preceding reductions in motor activity were noted in male rats during the fourth and eighth weeks and were attributed to the administration of m-Phenylenediamine; however, these observations were not considered neurotoxic effects but were considered indicative of general malaise in experimental animals. The general malaise was supported by concurrent clinical observations of lethargy. In female rats, the test substance did not induce changes in horizontal or vertical motor activity counts at any of the doses tested. Neuropathologic examinations did not indicate any abnormalities within the nervous system or skeletal muscle. It was concluded that m-Phenylenediamine was not neurotoxic and that the no observable effect level for this chemical in male and female rats was 5 mg/kg (Du Pont, 1992).

Reproductive Toxicity

The results of six reproductive toxicity studies are summarized in Table 7.

In the first study, the fetal toxicity of m-Phenylenediamine (99% pure) in water was evaluated using 125 female rats (weights = 200–250 g) that had been mated for the first time. The test substance was administered orally to three groups of 25 rats in doses of 10, 30, and 90 mg/kg/day, respectively, on gestation days 5 to 16. The doses were administered once daily at a dose volume of 10 mL/kg. The negative control group (25 rats) was dosed with water, and the positive control group (25 rats) with acetylsalicylic acid. Necropsy was performed at the end of the dosing period. Six rats in the highest (90 mg/kg/day) dose group died before day 20; these deaths were not considered test substance–related. On day 20 of gestation, dams in the 90-mg/kg/day and 30-mg/kg/day dose groups weighed less than those in the negative control and 10-mg/kg/day group. Feed consumption was also decreased in the 90-mg/kg and 30-mg/kg dose groups. Only the weight change for the highest-dose group was considered statistically significant. Areas of hepatic necrosis were noted in experimental and negative control groups at approximately the same frequency. These as well as other tissue changes noted at necropsy were considered unrelated to test sub-
### Table 7. Reproductive and developmental toxicity of m-Phenylenediamine

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Number of animals</th>
<th>Test procedure</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-Phenylenediamine (99% pure)</td>
<td>Three groups of 25 female rats (200–250 g, strain not stated)</td>
<td>Oral doses of 10, 30, and 90 mg/kg/day on gestation days 5–16</td>
<td>Strongly fetotoxic at highest dose; may be weakly teratogenic at doses high enough to be injurious to dam</td>
<td>SRC, 1981</td>
</tr>
<tr>
<td>m-Phenylenediamine (in propylene glycol)</td>
<td>Three groups of nine, eight, and seven female Sprague-Dawley rats, respectively (225–250 g)</td>
<td>Oral doses of 45, 90, and 180 mg/kg/day on gestation days 6–15</td>
<td>Numbers of fetal implantations and anomalies not significantly different from vehicle controls</td>
<td>Picciano et al., 1983</td>
</tr>
<tr>
<td>m-Phenylenediamine (in 2% solution of carboxymethylcellulose in distilled water)</td>
<td>30 female Sprague-Dawley rats</td>
<td>Oral doses of 35 mg/kg/day on gestation days 6–15</td>
<td>Not teratogenic at a dose that was sufficient to cause maternal toxicity</td>
<td>SRI International, 1984b</td>
</tr>
<tr>
<td>Oxidative hair dye formulation containing 1.5% m-Phenylenediamine</td>
<td>20 female Charles River CD rats</td>
<td>Dermal applications (2 mL/kg) to shaved skin on gestation days 1, 4, 7, 10, 13, 16, and 19</td>
<td>Not teratogenic nor embryotoxic</td>
<td>Burnett et al., 1976</td>
</tr>
<tr>
<td>Hair dye containing 1.5% m-Phenylenediamine mixed with equal volume of hydrogen peroxide</td>
<td>Nine groups of 40 Sprague-Dawley rats (20 males, 20 females per group mated initially; 6–8 weeks old)</td>
<td>Multigeneration reproduction study; prior to initial mating, dermal applications (0.5 mL, males and females) twice weekly up to 100 days old; number of animals mated reduced in subsequent generations</td>
<td>Fertility, gestation, survival, and live birth indices comparable among test and control groups</td>
<td>Burnett and Goldenthal, 1988</td>
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</tr>
<tr>
<td>Hair dye formulation containing 1.5% m-Phenylenediamine mixed with equal volume of hydrogen peroxide</td>
<td>34 female CD-1 (COBS) mice</td>
<td>Dermal applications (0.05 mL) to skin, clipped free of hair, twice weekly during 26-day premating period, days 1–12 of mating, and 18 days of gestation</td>
<td>Not teratogenic</td>
<td>Bio/dynamics, 1977</td>
</tr>
</tbody>
</table>
stance administration. Compared to the negative control group, the following statistically significant findings (significance limit of $P = 0.05$) were noted in the 90-mg/kg/day dose group: reduction in the number of litters with live pups; lower average placental weights; fewer total number of live pups; fewer live pups per litter; lower average body weight of a live pup, as well as an increase in the total resorptions; greater total number of late-dying embryos; greater total number of early-dying embryos; and higher percentage of dams with fetuses having minor alterations and greater frequency of fetuses having minor malformations. There were no major malformations. Statistically significant findings were not noted in fetuses of dams from any of the other experimental groups. It was concluded that m-Phenylenediamine was strongly fetotoxic and that it might be weakly teratogenic only with doses that are high enough to be injurious to the dam. The positive control, acetylsalicylic acid, was teratogenic (SRC, 1981).

In the second study, m-Phenylenediamine (in propylene glycol) was administered by gavage to 24 female Sprague-Dawley rats (weights = 225–250 g) on days 6 to 15 of gestation. The following doses were administered: 45 mg/kg (nine rats), 90 mg/kg (eight rats), and 180 mg/kg (seven rats). Solutions of m-Phenylenediamine were prepared daily and dosed at a rate of 10 mL/kg. Vitamin A and aspirin were administered to positive control groups on day 9 of gestation and days 6 to 15 of gestation, respectively. Vitamin A was administered at a dose of 100,000 IU per animal, and aspirin was administered at a dose of 350 mg/kg. All dams were killed by carbon dioxide inhalation on day 20 of gestation. There were no mortalities in experimental or vehicle control groups during the gestational period; however, a significant decrease in the mean maternal weight gain occurred (days 6–16 of gestation) in rats that received 180 mg/kg doses of m-Phenylenediamine. An increase in the number of fetal resorptions that was not statistically significant ($P = 0.05$) was also noted in the 180-mg/kg dose group. The numbers of fetal implantations and fetal anomalies in all experimental groups were not significantly different from those in the vehicle control group. A statistically significant increase in the number of abnormal fetuses with gross, soft tissue, and skeletal anomalies ($P = 0.001$) was observed in groups dosed with vitamin A or aspirin (Picciano et al., 1983).

In the third study, the reproductive toxicity of m-Phenylenediamine was evaluated using female Sprague-Dawley rats (weights = 200–300 g). The test substance (in a 2% solution of carboxymethylcellulose in distilled water) was administered by oral intubation to a group of 30 rats. Each animal received a single daily dose (35 mg/kg; dose volume = 10 mL/kg) of the test substance by gavage on days 6 through 15 of gestation. The vehicle control group was treated with 2% car-
boymethyl cellulose and the positive control group was treated with 200 mg/kg sodium salicylate. In statistical comparisons, the probability level for null hypothesis rejection was 0.05. Significant maternal body weight loss ($P < 0.01$) was noted within 3 days of the initiation of dosing. Maternal body weight gain was significantly reduced ($P < 0.01$) during the entire dosing period and during pregnancy. The incidence of resorbed litters and external or visceral malformations in the experimental group was not significantly different from that observed in the negative control group. The same was true regarding skeletal defects in the experimental group (incidence of rib malformations = 1–2%). In the positive control group, a significant increase in the number of resorbed litters ($P < 0.01$) and the average percentage of dead or resorbed fetuses per implant ($P < 0.01$) was noted. Minor rib malformations were observed in 14% of the positive control fetuses. The incidence of fetuses with any skeletal defect was statistically significant in the positive control group. It was concluded that m-Phenylene diamine was not teratogenic to rats at a dose that was sufficient to cause maternal toxicity (SRI International, 1984a).

In the fourth report, the fetal toxicity of an oxidative hair dye formulation containing 1.5% m-Phenylene diamine was evaluated using a group of 20 mated Charles River CD female rats. Three groups of 20 untreated rats of the same strain served as controls. The hair dye formulation was applied (dose = 2 mL/kg) to shaved skin of the dorsocapular area on days 1, 4, 7, 10, 13, 16, and 19 of gestation. The positive control group, 20 rats, received acetylsalicylic acid (250 mg/kg) by gavage on days 6 through 16 of gestation. All rats were killed by chloroform anesthesia on day 20 of gestation. Significant differences in the mean number of corpora lutea, implantation sites, and live fetuses or in the sex ratio were not observed between experimental and control groups. There were also no differences regarding the number of females with resorption sites or mean resorptions per pregnancy and soft-tissue anomalies. The number of experimental fetuses with accessory ribs was significantly ($P < 0.01$) different from observations in each of the three control groups; this was the only significant finding. Observations in the positive control group included increased teratogenicity and embryo death and decreased fetal weight. The authors concluded that the hair dye formulation did not produce embryotoxic or teratogenic effects (Burnett et al., 1976).

In the fifth study, the reproductive effects of a hair dye containing 1.5% m-Phenylene diamine were evaluated using 360 male and female Sprague-Dawley rats (6–8 weeks old). There were three untreated control groups. The experimental animals were divided into nine groups of 40, and a mixture of equal volumes of the dye and 6% hydrogen perox-
ide was applied to shaved skin of the back (dose = 0.5 mL twice weekly) until each rat was 100 days old. The rats were then paired and mated for 15 days; indices for live birth and survival of pups to weaning were calculated. The number of F₀ parents was reduced from 40 to 20 per group after the first (F₁a) litters had been weaned; the remaining animals were re-mated to produce F₁b at the end of a 10-day nontreatment period. Twenty male and 20 female rats per group, selected from the F₁b litters to become the F₁ parents of the next generation, received hair dye according to the same procedure that was described for the F₀ generation. At the end of 100 days, the rats were mated, avoiding brother–sister pairing, to produce the F₂a and F₂b litters. After the F₂b litters had been weaned, five female and five male F₁ parents were necropsied. Body weight gains, feed consumption, and survival were comparable among the test and control groups in each generation; the same was true for fertility, gestation, survival and live birth indices, and the mean numbers weaned and mean weaning weights for each litter. Mild dermatitis was observed intermittently throughout the treatment period in each generation of experimental animals; however, there were no pharmacotoxicologic signs observed that were related to application of the dye. Microscopic examination of tissues from five male and five female F₁b parental animals indicated no evidence of treatment-related lesions (Burnett and Goldenthal, 1988).

In the final study, the reproductive toxicity of a hair dye formulation containing 1.5% m-Phenylenediamine was evaluated using 34 sexually mature female CD-1 (COPS) mice and sexually mature male mice of the same strain. Initial body weights were not provided. Prior to testing, the hair dye formulation was mixed with an equal volume of 6% hydrogen peroxide. The animals were mated at the end of a 26-day premating treatment period. Treatment prior to mating involved application of the test substance to the back (clipped free of hair) at a dose volume of 0.05 mL per female mouse twice weekly. The test substance was also administered to female mice (same procedure) on days 1 to 12 of mating and during 18 days of gestation. Throughout the entire treatment period, application of the test substance was alternated between anterior and posterior halves of the clipped area in order to minimize skin irritation. Untreated mice (30 mice, backs clipped free of hair) served as controls. Necropsy was performed on all surviving females and fetuses delivered before or after day 18 of gestation. Only one of the experimental dams died; necropsy results were unremarkable. Pregnancy rates and mean numbers of implantations, live fetuses, and resorptions were comparable between experimental and control groups. Mean fetal weight in the experimental group was significantly greater (P < 0.05). The mean number of fetal ossification variations per fetus per litter in the experimental group was also sig-
nificantly greater ($P < 0.01$) than that observed in the control group. The ossification data were deemed suggestive of a retarding effect of the hair dye formulation on the ossification process, particularly in bones of the feet and the cervical and caudal vertebral centra. Eleven fetuses from the experimental group had feet missing. This finding was thought to have resulted from excessive maceration in potassium hydroxide solution in that no abnormalities involving the feet were noted at necropsy. None of the control mice had missing feet. The type and incidence of soft tissue malformations were considered similar between experimental and control groups and unremarkable. It was concluded that the hair dye formulation was not teratogenic (Bio/dynamics, 1977).

MUTAGENICITY

A variety of mutagenicity tests on m-Phenylendiamine have been conducted, with overall positive findings in the Ames test, with and without metabolic activation, but with mixed results in mammalian systems. The results of these studies are summarized in Table 8, but presentation of each study in the text is omitted in favor of a complete discussion of carcinogenicity studies in the next section.

CARCINOGENICITY

Twelve carcinogenicity studies are summarized in Table 9 and described in detail in the following paragraphs, except that no information additional to that in Table 9 was available regarding the work of Van Duuren (1980).

In a study by Amo et al. (1988), m-Phenylendiamine (0.04% and 0.02%) was administered in drinking water to two groups of 4-week-old female B6C3F1 mice (50 and 59 mice, respectively) and two groups of 4-week-old male B6C3F1 mice (50 and 56 mice, respectively) for 78 weeks, after which purified water was administered. Each mouse received 5 mL of the test solution daily, except on Saturday, when 10 mL was administered for 2 days. Dosages were increased to 6 mL per mouse at week 39, and then increased to 7 mL at week 45. The control group (50 female and 50 male mice) was given purified water. Two to seven mice from each group were killed a few weeks before the end of the treatment period, primarily due to morbidity from tumors; the remaining survivors were killed after 83 to 85 weeks. Survival rates at the end of the experiment were more than 86% in all groups. Tumors were observed in each group of mice; however, there were no significant increases in tumor incidence over that observed in the control group.
The incidences of hepatocellular tumors in all experimental groups were significantly lower than what was observed in the control group. Additionally, the incidences of hyperplastic liver nodules and lung adenomas were significantly lower than in the control group of male mice. The histologic features of the hepatocellular tumors were similar to those of spontaneous tumors that are found in aged B6C3F1 mice. The test substance did not induce lesions in any of the following organs and tissues that were examined microscopically: liver, lungs, spleen, bone marrow, lymph nodes, pancreas, kidneys, stomach, small and large intestines, heart, urinary bladder, thymus, adrenal glands, salivary glands, thyroid gland, brain, pituitary gland, ovaries, testes, sternum, and skin.

The carcinogenicity of m-Phenylenediamine was evaluated using 80 (40 male, 40 female) C3Hf/Bd mice and 40 (20 male, 20 female) C57BL/6Bd mice by Holland et al. (1979). C3Hf/Bd and C57BL/6Bd mice will be referred to as C3 and B6 mice, respectively. The male and female C3 mice were divided into two groups of 20, respectively; male and female B6 mice were divided into two groups of 10, respectively. The test substance (50 μL) was applied by micropipette to shaved dorsal skin on Mondays, Wednesdays, and Fridays, for 24 months or until the group’s skin tumor response exceeded 90%. The two groups of male C3 mice received a total of 0.6 mg and 3.0 mg of test substance per week, respectively, and the same was true for the two groups of female C3 mice. The four groups of B6 mice received the same quantity of test substance that was administered weekly to groups of C3 mice. The positive control, benzo(a)pyrene, was administered at doses of 0.1% and 0.01% (weight/vol) in both strains. Acetone-treated control groups (both strains) received 0.15 mg acetone per week. In C3 and B6 mice treated with benzo(a)pyrene, the skin tumor frequency reached nearly 100% during the first 12 months of exposure. m-Phenylenediamine did not induce skin tumors in either of the two strains of mice tested. Tumors were observed in other tissues of C3 and B6 mice dosed with m-Phenylenediamine; however, these incidences were not significantly different from those in the acetone-treated control groups.

In a study reported by ORNL (1981), the carcinogenicity of m-Phenylenediamine (93% pure) was evaluated using 25 male and 25 female C3Hf/Bd mice (10 weeks old). Initial body weights were not stated; however, during the sixth month, mean body weights for male and female rats were 32.2 and 27.1 g, respectively. The test substance, in acetone, was applied to shaved skin at concentrations of 1.5, 3.0, and 6.0 weight/vol % (total dose per week = 2.25, 4.5, and 9 mg, respectively) over a period of 24 months. Applications were made on Mondays, Wednesdays, and Fridays using a 50-μL pipette. Benzo(a)pyrene, positive control, was diluted to the extent that the level of response and dis-
<table>
<thead>
<tr>
<th>Test substance</th>
<th>Strain/cell type tested</th>
<th>Test procedure</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-Phenylenediamine (1–10,000 μg/plate)</td>
<td><em>Salmonella typhimurium</em> strains TA 97 and TA 98</td>
<td>Preincubation assay with and without metabolic activation (Haworth et al., 1983)</td>
<td>Overall results positive</td>
<td>Zeiger et al., 1988</td>
</tr>
<tr>
<td>m-Phenylenediamine (1–10,000 μg/plate)</td>
<td><em>Salmonella typhimurium</em> strains TA 98 and TA 100</td>
<td>Preincubation assay (Yahagi et al., 1975) and plate incorporation assay (Maron and Ames, 1983)</td>
<td>In both assays, mutagenic with metabolic activation and not mutagenic without metabolic activation</td>
<td>Gentile et al., 1987</td>
</tr>
<tr>
<td>m-Phenylenediamine (20–1000 μg/plate)</td>
<td><em>Salmonella typhimurium</em> strains TA 98 and TA 1538</td>
<td>Ames test (Ames et al., 1975) with and without metabolic activation</td>
<td>Strong mutagenic response with metabolic activation, weak mutagenic response without metabolic activation</td>
<td>Picciano et al., 1983</td>
</tr>
<tr>
<td>m-Phenylenediamine (unpurified and purified, 5–1000 μg/plate)</td>
<td><em>Salmonella typhimurium</em> strains TA 98, TA 100, and TA 1538</td>
<td>Ames test with and without metabolic activation</td>
<td>m-Phenylenediamine (purified and unpurified) highly mutagenic, dose response, with metabolic activation; without activation, purified form not mutagenic; unpurified form induced slight increase in number of revertants only in strain TA 1538</td>
<td>Shahin et al., 1980</td>
</tr>
<tr>
<td>Test substance</td>
<td>Strain/cell type tested</td>
<td>Test procedure</td>
<td>Results</td>
<td>Reference</td>
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<tr>
<td>m-Phenylendiamine (1-1000 µg/plate)</td>
<td><em>Salmonella typhimurium</em> strains TA 100, TA 1535, TA 1537, and TA 1538</td>
<td>Ames mutagenicity plate assay with and without metabolic activation</td>
<td>Mutagenic in strain TA 1538 (with metabolic activation)</td>
<td>Du Pont, 1975</td>
</tr>
<tr>
<td>m-Phenylendiamine (up to 500 µg/plate)</td>
<td><em>Salmonella typhimurium</em> strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538; <em>Saccharomyces cerevisiae</em> strain D4</td>
<td>Ames mutagenicity plate assay with and without metabolic activation</td>
<td>Mutagenic in strains TA 98, TA 100, TA 1537, and TA 1538 with metabolic activation; not mutagenic without activation, or in TA 1535 or <em>S. cerevisiae</em></td>
<td>Litton Bionetics, 1976</td>
</tr>
<tr>
<td>m-Phenylendiamine (500 µg/plate)</td>
<td><em>Salmonella typhimurium</em> strains TA 97, TA 1537, and TA 1538</td>
<td>Ames test with metabolic activation (Ames et al., 1975; Maron and Ames, 1983)</td>
<td>Mutagenic in all strains, the extent of differences in number of revertants between treated cultures and controls was related to the concentration of S9 in S9 mix</td>
<td>Shahin et al., 1985</td>
</tr>
<tr>
<td>m-Phenylendiamine (500 µg/plate)</td>
<td><em>Salmonella typhimurium</em></td>
<td>Ames test with and without metabolic</td>
<td>Potent mutagen with metabolic activation,</td>
<td>ORNL, 1978</td>
</tr>
<tr>
<td>Compound</td>
<td>Concentration</td>
<td>Strains/Details</td>
<td>Results</td>
<td>Reference</td>
</tr>
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<tr>
<td>m-Phenylenediamine (purified, 10–100 µg/plate)</td>
<td></td>
<td><em>Salmonella typhimurium</em> strains TA 98, TA 1537, and TA 1538</td>
<td>Activation (Ames et al., 1975)</td>
<td>de Giovanni-Donnelly, 1981</td>
</tr>
<tr>
<td>m-Phenylenediamine (0.2%)</td>
<td></td>
<td><em>Salmonella typhimurium</em> strains TA 1535, TA 1537, and TA 1538</td>
<td>Plate tests and suspension assays with and without metabolic activation</td>
<td>Litton Bionetics, 1974</td>
</tr>
<tr>
<td>m-Phenylenediamine (&lt; 1 mM)</td>
<td></td>
<td><em>Salmonella typhimurium</em> strains TA 98, TA 98/1,8-DNP₈, and YG 1024</td>
<td>Plant cell/microbe cocultivation assay with activation (Plewa et al., 1983, 1988)</td>
<td>Seo et al., 1993</td>
</tr>
<tr>
<td>m-Phenylenediamine (in urine fractions from Wistar rats that received 24 h dermal application of 4% aqueous m-Phenylenediamine)</td>
<td></td>
<td><em>Salmonella typhimurium</em> strain TA 98</td>
<td>Ames test (Ames et al., 1975)</td>
<td>Clemmensen et al., 1985</td>
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<tr>
<td>m-Phenylenediamine (500 µg/mL and 10 mg)</td>
<td></td>
<td><em>Saccharomyces cerevisiae</em> strains D3 and D4</td>
<td>Strain D3 tested with and without metabolic activation in liquid</td>
<td>Mayer and Goin, 1980</td>
</tr>
</tbody>
</table>

(Table continued on next page.)
Table 8. Mutagenicity of m-Phenylenediamine (continued)

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Strain/cell type tested</th>
<th>Test procedure</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-Phenylenediamine</td>
<td><em>Saccharomyces cerevisiae</em> strain D4</td>
<td>Suspension assays; strain D4 tested without activation in plate tests</td>
<td>(500 µg/plate); nonmutagenic in strain D3 with and without activation</td>
<td>Litton Bionetics, 1974</td>
</tr>
<tr>
<td>(2.5% and 5%)</td>
<td></td>
<td>Suspension assay with and without metabolic activation</td>
<td>Not mutagenic with or without activation</td>
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<tr>
<td>m-Phenylenediamine</td>
<td><em>Saccharomyces cerevisiae</em> strain D3</td>
<td>Suspension assay with and without metabolic activation</td>
<td>Not mutagenic with or without activation</td>
<td>Du Pont, 1975</td>
</tr>
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<td>(2%)</td>
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<tr>
<td>m-Phenylenediamine</td>
<td>Human lymphocytes</td>
<td>Chromosomal aberrations assay</td>
<td>Borderline mutagen; greatest number of chromosomal gaps noted at highest concentration</td>
<td>ORNL, 1978</td>
</tr>
<tr>
<td>(5, 50, and 200 µg/mL)</td>
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<tr>
<td>m-Phenylenediamine</td>
<td>Human lymphocytes</td>
<td>In vitro cytogenetics assay with and without metabolic activation</td>
<td>Not mutagenic with or without activation</td>
<td>Ho et al., 1978</td>
</tr>
<tr>
<td>(dose not stated)</td>
<td></td>
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<td>m-Phenylenediamine</td>
<td>Subclones of the CHO-K1-BH4 Chinese hamster ovary cell line</td>
<td>After incubation, mutagenicity measured by induction of 6-thioguanine resistance</td>
<td>Weak mutagen</td>
<td>ORNL, 1978</td>
</tr>
<tr>
<td>(≤ 500 µg/mL)</td>
<td></td>
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</tr>
<tr>
<td>m-Phenylenediamine</td>
<td>Chinese hamster ovary cells</td>
<td>In vitro cytogenetics assay with and without metabolic activation</td>
<td>Chromosomal aberrations induced without, but not with, metabolic activation;</td>
<td>National Toxicology Program, 1993</td>
</tr>
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<td>Substance</td>
<td>Organ/Species</td>
<td>Test Method</td>
<td>Result</td>
<td>Source</td>
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<tr>
<td>m-Phenylenediamine</td>
<td>L5178Y mouse lymphoma cells</td>
<td>Forward mutational assay, modification of procedure by Clive and Spector (1975)</td>
<td>Dose-related mutagenic response</td>
<td>Palmer et al., 1977</td>
</tr>
<tr>
<td>m-Phenylenediamine</td>
<td>Germ cells from 10-week-old hybrid C57BL/6J × DBA/2 male mice</td>
<td>Unscheduled DNA synthesis assay, Mice injected intraperitoneally with 50 and 100 mg/kg m-Phenylenediamine</td>
<td>Not mutagenic</td>
<td>Tanaka and Katoh, 1979</td>
</tr>
<tr>
<td>m-Phenylenediamine</td>
<td>E. coli WP2s (λ)</td>
<td>Microscreen assay; lambda phage induction in E. coli used as endpoint. Induction of lambda phage results from derepression of set of coordinately regulated genes that become activated when DNA is damaged</td>
<td>Mutagenic</td>
<td>Rossman et al., 1991</td>
</tr>
<tr>
<td>m-Phenylenediamine</td>
<td>Groups of 15 Holtzman male albino rats</td>
<td>Dominant lethal test; male rats dosed intraperitoneally three times per week for 10 weeks and then mated with females</td>
<td>m-Phenylenediamine (98% pure) weakly positive in initial test: numbers of dead implants per pregnant female in 12.5 and 50 mg/kg dose groups significantly</td>
<td>Sheu and Green, 1979</td>
</tr>
</tbody>
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Table 8. Mutagenicity of m-Phenylenediamine (continued)

<table>
<thead>
<tr>
<th>Test substance</th>
<th>Strain/cell type tested</th>
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<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-Phenylenediamine (20 mg/kg)</td>
<td>20 male Charles River CD rats</td>
<td>Dominant lethal test; Male rats dosed intraperitoneally three times per week for 8 weeks and then mated with females</td>
<td>Increased over that in DMSO control group; m-Phenylenediamine (&gt; 99% pure) did not induce dominant lethality in retest</td>
<td>Burnett et al., 1977</td>
</tr>
<tr>
<td>m-Phenylenediamine (56.7% weight % in mixture; doses = 20, 40, and 80 mg/kg/day)</td>
<td>Groups of 25 male Sprague-Dawley rats</td>
<td>Dominant lethal test; Male rats received oral doses daily for 13 weeks; dosing followed by mating with females</td>
<td>No evidence of increase in postimplantation loss that could indicate a dominant lethal effect</td>
<td>SRI International, 1984b</td>
</tr>
</tbody>
</table>
distribution of time to skin tumor appearance would be similar to that of a weak skin carcinogen. The positive control was tested at concentrations of up to 0.01 weight/vol % In a preliminary toxicity evaluation in which m-Phenylenediamine was applied to the skin of rats daily for 2 weeks at a concentration of 12%, the test substance was not a primary skin irritant but was systemically toxic. It was concluded that m-Phenylenediamine did not induce skin tumors when administered at concentrations of 1.5, 3.0, or 6.0 weight/vol % benz(a)pyrene, positive control, induced skin tumors.

Burnett et al. (1975) evaluated the carcinogenicity of an experimental hair dye formulation containing m-Phenylenediamine using two groups of 50 male and two groups of 50 female random-bred Swiss Webster mice (6–8 weeks old). The composition of the hair dye formulation was as follows: m-Phenylenediamine base (0.17%), resorcinol (0.40%), p-Phenylenediamine (1.50%), 2,5-toluenediamine sulfate (3.0%), ammonia (1.74%), sodium sulfate (0.20%), isopropanol (3.0%), oleic acid (5.0%), and deionized water (80.7%). The formulation was mixed with an equal volume of 6.0% hydrogen peroxide, and 0.5 mL of the mixture was applied to shaved skin of the midscapular region once weekly (two groups, male and female) and once every other week (two groups) for a total of 18 months. Four groups of 50 control mice (vehicle control groups) were treated with a base containing no added dye intermediate according to the same procedure. The two positive control groups (50 males, 50 females) were dosed with 7,12-dimethylbenz[a]anthracene (DMBA) in acetone. The concentration of the positive control was adjusted such that 0.05 mL provided a weekly dosage of 50 ng DMBA for 6 months, 10 μg/wk for 4 months, and 50 μg/wk for the remainder of the study. No signs of systemic toxicity were observed in the experimental groups, and all of the hematologic data recorded were within normal limits. The following disease processes commonly observed in aged random-bred Swiss Webster mice occurred in all groups and at the same frequency: chronic murine pneumonia; amyloidosis; chronic focal nephritis; focal lymphoid infiltration in the lungs, liver, and kidneys; and testicular atrophy. The incidence of alveologenic adenomas and adenocarcinomas was comparable among test and control groups and was within the range of control values for the strain of mice that was tested. The authors concluded that the hair dye formulation was not carcinogenic (Burnett et al., 1975). For this study, the IARC Working Group noted the high incidence of tumors in mice treated with the dye base alone, which made up the greatest part of the hair dye formulation (IARC, 1978).

In later work, Burnett et al. (1980) evaluated the carcinogenicity of an oxidative hair dye formulation containing 1.5% m-Phenylenediamine using random-bred Swiss Webster mice (50 males, 50 females; 6–8
<table>
<thead>
<tr>
<th>Test substance</th>
<th>Animals tested</th>
<th>Test procedure</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-Phenylenediamine</td>
<td>Male and female mice (number and strain not stated)</td>
<td>72-week oral feeding study (No further details reported)</td>
<td>Not carcinogenic</td>
<td>Van Duuren, 1980</td>
</tr>
<tr>
<td>m-Phenylenediamine (0.04% and 0.02% in drinking water)</td>
<td>Two groups of 4-week old female B6C3F1 mice (59 and 50, respectively). Two groups of 4-week-old male mice (56 and 50, respectively)</td>
<td>Administered in drinking water for 78 weeks; test solution volume increased from 5 mL-6 mL at week 39, and to 7 mL at week 45</td>
<td>No significant increases in tumor incidence over that observed in the control group</td>
<td>Amo et al., 1988</td>
</tr>
<tr>
<td>m-Phenylenediamine</td>
<td>40 male and 40 female C3Hf/Bd mice (two groups of 20 per sex); 20 male and 20 female C57BL/6Bd mice (two groups of 10 per sex)</td>
<td>Test substance (50 µL) applied by micropipette to shaved dorsal skin on Mondays, Wednesdays, and Fridays for 24 months or until group's tumor response exceeded 90%</td>
<td>No skin tumors; tumor incidence in other tissues not significantly different from acetone-treated controls</td>
<td>Holland et al., 1979</td>
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<tr>
<td>m-Phenylenediamine (1.5, 3.0, and 6.0 weight/vol %)</td>
<td>25 male and 25 female C3Hf/Bd mice (10 weeks old)</td>
<td>Test substance applied by micropipette to shaved skin on Mondays, Wednesdays, and Fridays for 24 months</td>
<td>No skin tumors</td>
<td>ORNL, 1981</td>
</tr>
<tr>
<td>Compound</td>
<td>Experimental Details</td>
<td>Outcome Description</td>
<td>Reference</td>
<td></td>
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<td>--------------------------------</td>
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<tr>
<td>Oxidative hair dye formulation containing 1.5% m-Phenylenediamine</td>
<td>50 male and 50 female random-bred Swiss Webster mice from the Eppley colony Formulation mixed with equal volume of hydrogen peroxide and applied (0.5 mL) to the interscapular region once weekly for 21 months</td>
<td>Not carcinogenic</td>
<td>Burnett et al., 1980</td>
<td></td>
</tr>
<tr>
<td>Hair dye formulation containing 0.17% m-Phenylenediamine</td>
<td>Two groups of 50 male and two groups of 50 female random-bred Swiss Webster mice (6–8 weeks old) Formulation mixed with equal volume of 6% hydrogen peroxide; 0.5 mL of mixture applied to shaved skin of back once weekly (two groups, male and female) and once every other week (two groups) for 18 months</td>
<td>Hair dye formulation not carcinogenic; incidence of alveologic adenomas and adenocarcinomas comparable among test and control groups and within range of control values for strain tested</td>
<td>Burnett et al., 1975</td>
<td></td>
</tr>
<tr>
<td>m-Phenylenediamine</td>
<td>Male rats (number and strain not stated) 72-week oral feeding study (No further details reported)</td>
<td>Not carcinogenic</td>
<td>Van Duuren, 1980</td>
<td></td>
</tr>
<tr>
<td>Hair dye containing 1.5% m-Phenylenediamine</td>
<td>60 male and 60 female weanling Sprague-Dawley rats Mixture of equal volumes of the dye and 6% hydrogen peroxide applied to shaved skin of the back (dose = 0.5 mL) twice weekly for 2 years</td>
<td>Compared to the third control group (no adenomas observed), the incidence of adenocarcinoma/carcinoma in the mammary glands of experimental animals was significantly lower</td>
<td>Burnett and Goldenthal, 1988</td>
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</tr>
</tbody>
</table>

(Table continued on next page.)
<table>
<thead>
<tr>
<th>Test substance</th>
<th>Animals tested</th>
<th>Test procedure</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-Phenylenediamine</td>
<td>Two groups of five Wistar King rats (sex not stated)</td>
<td>Doses of 9 and 18 mg/kg, respectively, injected subcutaneously on alternate days for 5 months</td>
<td>Fibrosarcomas observed in one of five rats dosed with 18 mg/kg; no tumors in 9-mg/kg dose group</td>
<td>Saruta et al., 1962</td>
</tr>
<tr>
<td>m-Phenylenediamine hydrochloride</td>
<td>Two groups of five Wistar King rats (sex not stated)</td>
<td>Doses of 12 and 24 mg/kg, respectively, injected subcutaneously on alternate days for 5 months</td>
<td>No tumors in either dose group</td>
<td>Saruta et al., 1962</td>
</tr>
<tr>
<td>m-Phenylenediamine 2HCl</td>
<td>Two groups of 25 male weanling Charles River CD rats (4–6 weeks old)</td>
<td>The rats were fed m-Phenylenediamine 2HCl, in the diet, at a dose of 2000 mg/kg (maximum tolerated dose) for 18 months</td>
<td>Not carcinogenic</td>
<td>Weisburger et al., 1978</td>
</tr>
<tr>
<td>Compound</td>
<td>Description</td>
<td>Carcinogenicity</td>
<td>Reference</td>
<td></td>
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</tr>
<tr>
<td>m-Phenylenediamine 2HCl</td>
<td>Two groups of 25 male and two groups of 25 female random-bred albino CD-1 mice</td>
<td>Not carcinogenic</td>
<td>Weisburger et al., 1978</td>
<td></td>
</tr>
<tr>
<td></td>
<td>One group of female rats was fed m-Phenylenediamine 2HCl, in the diet, at a dose of 4000 mg/kg (maximum tolerated dose) for 18 months; the remaining three groups were fed 2000 mg/kg for 18 months</td>
<td></td>
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</tbody>
</table>
weeks old) from the Eppley colony. Two groups of mice (50 males, 50 females each) served as untreated controls. The hair dye formulation was mixed with an equal amount of hydrogen peroxide, and the dye mixture was applied to a single site (approximately 1 cm², clipped free of hair) in the interscapular region of each animal. Applications (0.5 mL/application) were made once weekly for 21 months. Skin lesions were charted weekly, and tumors were classified as benign or malignant at histopathologic evaluation. At the end of the study, gross and microscopic examinations were performed on moribund animals as well as those that were found dead. Prior to termination of the study, 10 males and 10 females from each experimental and control group were killed after 7 and 9 months of treatment. Necropsy was performed on these animals and organ-to-body weight ratios calculated. Of the 50 male and 50 female experimental mice, 9 males and 13 females survived the 21-month treatment period. None of the differences in organ weight (liver or kidneys)-to-body weight ratios represented significant departures from control values. Skin tumors were diagnosed in experimental and control groups; however, the skin tumor incidence was low and not considered treatment related. Liver hemangioma, lung adenoma, and malignant lymphoma are the predominant tumor types in the Eppley colony, and the high spontaneous incidence of malignant lymphomas is characteristic of the Eppley Swiss colony. A statistical analysis (Fisher's exact test) of the distribution of these three tumor types among control and experimental groups indicated that none of the chi-square values was significant at $P = 0.05$. Thus, the oxidative hair dye formulation containing 1.5% m-Phenylenediamine was not considered carcinogenic.

In a study reported by Burnett and Goldenthal (1988), the carcinogenicity of a hair dye containing 1.5% m-Phenylenediamine was evaluated using 60 male and 60 female weanling Sprague-Dawley rats that were randomly selected from each group of F₁a litters produced in a multigeneration reproduction study. There were three untreated control groups. The multigeneration reproduction study is summarized in the section on reproductive effects. A mixture of equal volumes of the dye and 6% hydrogen peroxide was applied to shaved skin of the back (dose = 0.5 mL twice weekly) for 2 years. After approximately 12 months of treatment, five male and five female rats from each group were killed and necropsied; representative tissue samples were collected. Rats that died or were killed in extremis during the study or at the end of the study (week 117) were also necropsied. Changes in body weight were similar for both the control and treated rats. A wide variety of non-neoplastic lesions was noted in control and experimental animals. These were considered to be spontaneous lesions that are commonly found in aging Sprague-Dawley rats. The most common tumors (those seen in
more than five animals in a group) were found in the pituitary gland, adrenal glands, liver, pancreas, mammary glands, uterus, thyroid gland, and hemopoietic system. Compared to the third control group (no adenomas observed), the incidence of adenocarcinoma or carcinoma in the mammary glands of experimental animals was significantly increased (only significant finding in tumor-bearing organs); however, this was not true when the incidence was compared with that in the other two control groups.

In a study by Saruta et al. (1962), the carcinogenicity of m-Phenylene diamine and m-Phenylene diamine hydrochloride was evaluated using four groups of five Wistar-King rats (sex not stated); both test substances were dissolved in 0.5 mL of distilled water. In the two high-dose groups, m-Phenylene diamine (dose = 18 mg/kg) and m-Phenylene diamine hydrochloride (dose = 24 mg/kg), respectively, were injected subcutaneously into the back on alternate days for 5 months. Animals in the lower-dose groups received subcutaneous injections of m-Phenylene diamine (9 mg/kg) and m-Phenylene diamine hydrochloride (12 mg/kg), respectively, on alternate days for 11 months. Two control groups of rats were injected with 0.5 mL of distilled water for either 5 or 11 months. Fibrosarcomas were observed in one of five rats in the m-Phenylene diamine low-dose group and in one of five rats in the m-Phenylene diamine hydrochloride high-dose group. Tumors were not observed in any of the remaining experimental or control groups. With reference to the carcinogenicity studies by Saruta et al. (1962) and Burnett et al. (1975), the IARC Working Group stated that no evaluation of the carcinogenicity of the m-Phenylene diamine or m-Phenylene diamine hydrochloride could be made (IARC, 1978, 1987).

Weisburger et al. (1978) evaluated the carcinogenicity of m-Phenylene diamine 2HCl using two groups of 25 male weanling Charles River CD rats and random-bred male and female albino CD-1 mice (two groups of 25 per sex); mice and rats were 4 to 6 weeks old. The animals were fed m-Phenylene diamine 2HCl, in the diet, at the maximum tolerated dose (25 male rats, 25 male mice, and 25 female rats) and at half of this dose (remaining three groups) for a total of 18 months. The maximum tolerated dose was a 2000 mg/kg diet for male rats and a 4000 mg/kg diet for male and female mice. After 18 months of feeding, mice and rats were fed a basal diet of Purina laboratory chow (control diet) for 3 and 6 months, respectively, prior to termination of the study. This diet was also fed to untreated control groups (25 male rats, 25 male mice, 25 female mice) for 18 months. Necropsy was performed on all animals that died after 6 months of feeding or that were killed at the end of the study. A high spontaneous tumor incidence was noted in control rats and mice, and similar observations have been reported in earlier studies involving the strains tested (Homburger et al., 1975;
MacKenzie and Garner, 1973; Prejean et al., 1973; Sher, 1974). The authors concluded that m-Phenylenediamine 2HCl was inactive in all groups of animals tested in this study.

**CLINICAL ASSESSMENT OF SAFETY**

**Absorption and Excretion**

Each of three subjects (weights not stated) ingested 0.5 g of m-Phenylenediamine hydrochloride and collected urinary specimens at the end of 15 min and 1, 4, and 12 h. Griess' m-Phenylenediamine-nitrite reaction was used for the detection of m-Phenylenediamine in the urine. In this test, the production of urine with an intense yellowish brown to deep brown (Bismarck brown) color indicated the presence of m-Phenylenediamine. Urine with an unmistakable brown color was obtained from two subjects, and only at 4 and 12 h postingestion. These data include that m-Phenylenediamine was absorbed to some extent. The authors stated that the observation that specimens from both subjects were darker in color than those obtained at 15 min and 1 h indicated that an oxidation product, quinonediimine, was present (Hanzlik, 1922).

**Skin Sensitization**

One of 150 subjects was primarily sensitive to m-Phenylenediamine, and four of 150 to both m-Phenylenediamine and p-Phenylenediamine. Additionally, 14 of the subjects were sensitive to p-Phenylenediamine and none was sensitive to o-Phenylenediamine. The experimental protocol and ages of the subjects tested were not stated (GE, 1983).

**Occupational Exposure**

An evaluation of workers (30–50 years old; number not stated) who came in contact with m-Phenylenediamine during its production was performed; the duration of potential exposure was 5 to 10 years. Dysuria was experienced by 13.4% of the workers. Additionally, the results of a scratch test on m-Phenylenediamine allergen were positive in 8% of the workers. Eosinophiluria was also noted in these workers (8%), and 0.3 to 40 μg m-Phenylenediamine per 100 mL of urine was detected. Mucosal edema, polypos swelling, and infiltration in the area of the urinary bladder triangle and cervix were identified by cystoscopy, and the eosinophilic character of each was confirmed cytologically (IARC, 1978).

A total of 38 patients (ages not stated) with dermatitis were exposed to m-Phenylenediamine and epoxy resin in the workplace. The results
of sensitization tests (procedure not stated) indicated eight positive reactions to m-Phenylene diamine; the remaining 30 subjects tested negative. The 8 subjects with positive reactions and the 30 subjects with negative reactions had been exposed to m-Phenylene diamine for 20 months and 14.5 months, respectively, prior to developing dermatitis. Patients with negative reactions to m-Phenylene diamine had positive reactions to epoxy resin (GE, 1983).

Hyperreflexia, hyporeflexia, anisoreflexia, skin hyperesthesia, and pathologic changes in the kidneys and liver were observed in humans professionally exposed to m-Phenylene diamine at doses of 1 μg/L to 2 μg/L. Neither the number of individuals involved nor the duration of exposure was stated (GE, 1983).

Antibodies (specific types not stated) to m-Phenylene diamine were detected in the blood of individuals exposed to m-Phenylene diamine while working with epoxy resin hardeners. Neither the number of workers involved nor the duration of exposure was stated (GE, 1983).

**EPIDEMIOLOGY**

Between 35% and 45% of American women dye their hair, often at monthly intervals, over a period of years (CTFA, 1993). This estimate is drawn from market research data on hair dye product use, generally from women aged 15 to 60.

A number of epidemiologic studies have investigated the association between cancer and occupation as a hairdresser or barber, or between cancer and personal use of hair dyes. The World Health Organization's IARC empaneled a Working Group on the Evaluation of Carcinogenic Risks to Humans to review all available data on these issues. The Working Group met October 6–13, 1992, in Lyon, France (IARC, 1993).

The charge to the IARC Working Group was to ascertain that all appropriate data had been collected and were being reviewed, to evaluate the results of the epidemiologic and experimental studies and prepare accurate summaries of the data, and to make an overall evaluation of the carcinogenicity of the exposure to humans.

The IARC Working Group concluded that: “There is inadequate evidence that personal use of hair colourants entails exposures that are carcinogenic.” Hence, “Personal use of hair colourants cannot be evaluated as to its carcinogenicity (Group 3).” The IARC Working Group also concluded that: “There is limited evidence that occupation as a hairdresser or barber entails exposures that are carcinogenic.” Hence, “Occupation as a hairdresser or barber entails exposures that are probably carcinogenic (Group 2A)” (IARC, 1993). The Expert Panel concludes that the relevance of the occupational data and conclusion to individuals using hair dyes is unclear.
SUMMARY

Both m-Phenylenediamine and m-Phenylenediamine Sulfate are aromatic amines. Their molecular weights are 108.15 and 206.21, respectively. m-Phenylenediamine is soluble in alcohol, ether, and water and is produced by the reduction of metadinitrobenzene or nitroaniline with iron and hydrochloric acid. The sulfate may be prepared by reaction of the appropriate amine with sulfuric acid.

m-Phenylenediamine and m-Phenylenediamine Sulfate are used as hair colorants in 162 and 28 hair dye products, respectively. Both ingredients have been used in hair dyes at concentrations of up to 1%. Data submitted to CTFA in 1995 indicated that m-Phenylenediamine and m-Phenylenediamine Sulfate were used in hair colors at concentrations of up to 3%.

The percutaneous absorption of m-Phenylenediamine has been demonstrated in dogs (hydrochloride salt in gel) and rats (14C-m-Phenylenediamine in saline). The principal route of excretion of percutaneously absorbed m-Phenylenediamine in rats was in the urine; the following three urinary metabolites were also identified: N-acetyl-1,3-diaminobenzene, N,N'-diacetyl-2,4-diaminophenol, and N,N'-diacetyl-1,3-diaminobenzene. m-Phenylenediamine has also been detected in the urine of humans following the ingestion of m-Phenylenediamine hydrochloride.

Nine of 10 rats exposed (inhalation) to m-Phenylenediamine distilled flakes for 6 h died within 24 h to 48 h after the end of exposure. Pulmonary congestion was noted in all 10 animals at necropsy. In another study, the mean acute inhalation LC50 (groups of 10 rats, 4-h exposure) for m-Phenylenediamine was 3.2 mg/L.

The acute oral LD50 for m-Phenylenediamine (in oil-in-water emulsion) in a group of 10 rats was 650 mg/kg. In other tests involving m-Phenylenediamine distilled flakes (20% aqueous solution), the acute oral LD50 in groups of five rats was 360 mg/kg, and the minimal lethal dose in a group of six rabbits was in the range of 250 mg/kg to 500 mg/kg.

Minimal nephrosis was the only pathologic change noted in six rats dosed orally with m-Phenylenediamine (3% suspension in peanut oil; dose = 90 mg/kg/day) over a 2-week period. There were not test substance-related pathologic changes in six rats dosed (intragastric intubation) with 0.8% m-Phenylenediamine (in mixture; 134 mg/kg/day) over a 2-week period.

In a subchronic (90-day) oral toxicity study involving groups of 20 rats, the no-effect level was 6 mg m-Phenylenediamine/kg body weight. At histopathologic examination, degenerative lesions in the liver were observed only in the 18-mg/kg/day dose group. There was no indication of toxic injury to the kidneys.
m-PHENYLENEDIAMINE AND m-PHENYLENEDIAMINE SULFATE

The dermal application of undiluted m-Phenylenediamine to two pairs of rabbits (doses of 200 mg/kg and 2000 mg/kg, respectively) did not result in death, and the test substance was classified as nontoxic. At necropsy, hepatorenal toxicity (pale and swollen livers and kidneys) was noted in animals from groups of five mice that received daily applications of m-Phenylenediamine in acetone. Concentrations of 10% and greater were applied over a 2-week period.

There was no evidence of test substance-induced toxicity in 12 rabbits that received dermal applications (1 mL/kg) of an oxidative dye formulation containing 1.5% m-Phenylenediamine for 13 weeks. The dye was mixed with an equal volume of hydrogen peroxide prior to application.

At most, m-Phenylenediamine was classified as a moderate ocular irritant in albino rabbits. m-Phenylenediamine (as supplied) was classified as a corrosive material in rabbits; however, in another study, undiluted m-Phenylenediamine was not a primary skin irritant.

m-Phenylenediamine (25% and 35% aqueous solutions) induced irritation and sensitization reactions in albino guinea pigs. When guinea pigs were tested with lower concentrations of m-Phenylenediamine (0.1%–10.0%), skin irritation was noted at the highest concentration. Mild and no sensitization were noted at concentrations of 1.0% and 0.1%, respectively. There was no evidence of cross-sensitization in guinea pigs sensitized with p-Phenylenediamine and challenged with 1% m-Phenylenediamine in another study.

m-Phenylenediamine was not neurotoxic when administered orally to rats at doses of up to 20 mg/kg for 90 days.

When administered orally to female rats at doses of up to 90 mg/kg/day on gestation days 5 to 16, m-Phenylenediamine was strongly fetotoxic at the highest dose but not teratogenic at any of the doses tested. m-Phenylenediamine also was not teratogenic when administered on gestation days 6 to 15 at doses of up to 180 mg/kg/day. Oxidative hair dye formulations containing 1.5% m-Phenylenediamine were not teratogenic in rats or mice.

In most of the Ames mutagenicity assays, m-Phenylenediamine was mutagenic to Salmonella typhimurium strains with, but not without, metabolic activation. In human lymphocyte cultures, m-Phenylenediamine was classified as a borderline mutagen in the chromosomal aberrations assay and was not mutagenic in the cytogenetics assay (with or without activation). Positive and negative responses were also noted in a variety of other in vitro and in vivo mutagenicity tests.

In studies involving mice and rats, neither m-Phenylenediamine nor hair dye formulations containing this ingredient were carcinogenic. m-Phenylenediamine was tested in oral and dermal carcinogenicity studies, whereas the hair dye formulations were tested only in dermal car-
cinogenicity studies. Fibrosarcomas were observed in one of five rats injected subcutaneously with m-Phenylenediamine over a period of 5 months; tumors were not observed in a second group dosed with 9 mg/kg.

m-Phenylenediamine-induced sensitization was noted in 1 of 15 patients. Four of the subjects were sensitive to m-Phenylenediamine and p-Phenylenediamine.

Positive scratch test results were reported for 8% of the workers (number not stated) who came in contact with m-Phenylenediamine during its production over a period of 5 to 10 years. In another study, 8 of 38 dermatitis patients who had been exposed to m-Phenylenediamine in the workplace had sensitization reactions to this ingredient. The 8 patients with positive reactions and the remaining 22 (negative reactions) had been exposed over periods of 20 and 14.5 months, respectively.

Hyperreflexia, hyporeflexia, skin hyperesthesia, and pathologic changes in the kidneys and liver were observed in humans (number not stated) professionally exposed to m-Phenylenediamine at doses of 1 to 2 μg/L. The duration of exposure was not stated.

DISCUSSION

The results of a skin irritation and sensitization test indicated that 10% m-Phenylenediamine induced skin irritation (intact skin) in four of nine guinea pigs tested and that repeated applications of 1% m-Phenylenediamine, followed by challenge at the same concentration, caused only mild sensitization in one animal; however, in a skin irritation test involving 10 guinea pigs (intact skin), the results were negative at both test concentrations of m-Phenylenediamine (10% and 50%) in a hydrophilic ointment vehicle. Given these test results, the Expert Panel determined that m-Phenylenediamine and m-Phenylenediamine Sulfate can be used safely in hair dyes at concentrations of up to 10%.

The Panel noted the mixed results reported in in vitro mutagenicity tests on m-Phenylenediamine; however, the critical endpoints of carcinogenicity and teratogenicity were negative.

CONCLUSION

On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that m-Phenylenediamine and m-Phenylenediamine Sulfate are safe for use in hair dyes at concentrations of up to 10%.
REFERENCES


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CIR Panel Book Page 76

CTFA. 1995. Submission of unpublished data by CTFA. Use levels for various ingredients including m-Phenylenediamine and m-Phenylenediamine sulfate. Unpublished data submitted by CTFA.*


m-PHENYLENEDIAMINE AND m-PHENYLENEDIAMINE SULFATE


Food and Drug Administration (FDA). 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). Federal Register 44:59509–59510.


IARC. 1993. Occupational Exposures of Hairdressers and Barbers and Personal Use of Hair Colourants; Some Hair Dyes, Cosmetic Colourants,


m-PHENYLENEDIAMINE AND m-PHENYLENEDIAMINE SULFATE


m-PHENYLENEDIAMINE AND m-PHENYLENEDIAMINE SULFATE


*Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Washington, DC 20036.
Memorandum

TO: F. Alan Andersen, Ph.D.
   Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
   Industry Liaison to the CIR Expert Panel

DATE: October 31, 2012

SUBJECT: Concentration of Use by FDA Product Category: m-Phenylenediamine and m-
   Phenylendiamine Sulfate
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>FDA Code†</th>
<th>Product Category</th>
<th>Maximum Concentration of Use</th>
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<td>m-Phenylenediamine</td>
<td>06A</td>
<td>Hair dyes and colors (all types requiring caution statement and patch test)</td>
<td>0.01-0.2%</td>
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<tr>
<td>m-Phenylenediamine Sulfate</td>
<td>06A</td>
<td>Hair dyes and colors (all types requiring caution statement and patch test)</td>
<td>1%</td>
</tr>
</tbody>
</table>

†Product category codes used by FDA

Information collected in 2012
Table prepared October 25, 2012