Hair Dye Package:
Acid Orange 3;
N,*N*-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate;
6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Chloro-2-Aminophenol

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, Senior Scientific Writer/Analyst, CIR
Date: May 23, 2022
Subject: Re-Review of 3 Hair Dye Ingredient Reports

Enclosed is the re-review package of 3 hair dye ingredient reports. Because it has been at least 15 years since these reports were published, in accord with Cosmetic Ingredient Review (CIR) Procedures, the Panel should consider whether these safety assessments should be re-opened. Below, you will find summarized information on Acid Orange 3, *N*,*N*-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate, and 6 cresol-related hair dyes (6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Chloro-2-Aminophenol). The Panel should carefully review the historical overview, comparison of original and new use data, the search strategy used, a synopsis of notable new data for each ingredient or ingredient group (*newdata_HairDyeRR_062022*), and the use table (*usetable_HairDyeRR_062022*). If upon review of the new studies and updated use data the Panel determines that a re-review is warranted, a draft amended report will be presented at an upcoming meeting.

This is a new format for approaching the backlog of rereviews on the Panel's docket. In each case, the Panel is only being asked to decide if the report should be reopened or not.

Acid Orange 3

The original review of Acid Orange 3 was published in 2000 with the conclusion that it is "safe for use in hair dye formulations at concentrations ≤ 0.2%." (The original review is included in this report package as *originalreport-AcidOrange3_HairDyeRR_062022*).

When the report was published, the number of reported uses in hair dye formulations was 4. During the writing of the original safety assessment, the FDA had discontinued providing concentration of use data and the Council was not yet providing it to CIR at that time. The data obtained was from a 1984 FDA survey which provided the concentrations in range groupings with numbers of formulations. In 1984, 33 out of 34 formulations reported that Acid Orange 3 was used at up to 1% and 1 formulations reported that it was used between 10% and 25%. The FDA VCRP database in 2022 reported that Acid Orange 3 has one use in a nail polish and enamel. A survey performed by the Council in 2022 had no reported concentrations of use.

In a search of available published literature for studies dated 1997 forward, a dermal sensitization study (Buehler guinea pig sensitization test with induction of 10% Acid Orange 3 in propylene glycol and challenges at 2.5%, 5%, or 10%.) reported that Acid Orange 3 was positive for allergic reactions. Additionally, the European Commission (2004 SCCNFP opinion) determined that Acid Orange 3 could not be considered safe for hair dying purposes due to the lack of an adequate safety dossier. At the time the original report was written, there were no restrictions on the use of Acid Orange 3 in cosmetics in Europe; however, European regulations regarding cosmetic ingredients now categorize Acid Orange 3 in Annex II, the list of substances prohibited in cosmetic products in Europe.

***N*,*N*-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate**

The original review for *N*,*N*-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate was published in 1992 with the conclusion that it is "safe as a cosmetic ingredient in present practices of use and concentration." (The original review is included in this report package as *originalreport-NNBis_HairDyeRR_062022*).

When the report was published, the number of reported uses in hair dye formulations was 183. The FDA reported that it was used at concentrations of ≤ 5%. The FDA VCRP database in 2022 has reported an increase in uses to a total of 193. A survey performed by the Council in 2022 reported the maximum use concentration range to be 0.006% to 1.3% in hair dye formulations.

In a search of available published literature for studies dated 1990 forward, a dietary carcinogenicity study in rats was performed and concluded that *N*,*N*-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate was not carcinogenic. Additionally, the European Commission (2006 SCCP opinion) determined that *N*,*N*-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate as an oxidative hair dye at a maximum concentration of 2.5% in finished products "does not pose a risk to the health of the consumer." The European Commission further advised that this hair dye ingredient is a tertiary amine that is prone to nitrosation and should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb. The 2006 SCCP opinion contains dermal absorption data and toxicological data that were not available when the original report was written, and includes NOAELs for repeated dose and DART studies, in vitro and in vivo genotoxicity data (mixed results in vitro, negative in vivo), and dermal carcinogenicity data (negative when tested up to 0.5% with and without hydrogen peroxide). At the time the original report was written, there were no restrictions on the use of *N*,*N*-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate in cosmetics in Europe; however, European regulations regarding cosmetic ingredients now categorize this hair dye ingredient in Annex III, the list of substances which cosmetic products must not contain except subject to the restrictions laid down (oxidative hair dye substance with a maximum concentration applied after mixing not exceeding 2.5%).

6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Chloro-2-Aminophenol

The original review for 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Chloro-2-Aminophenol was published in 2004 with the following conclusion:

> "The CIR Expert Panel concludes that the available data support the safety of 6-Amino-*m*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol as used in oxidative and nonoxidative (semipermanent) hair dyes. The available data also support the safety of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol for use in oxidative hair dyes, but are insufficient to support the safety of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol in nonoxidative (semipermanent) hair dyes."

(The original review is included in this report package as *original report-AminoCresols_HairDyeRR_062022*).

When the report was published, the only ingredient with uses reported was 6-Amino-*m*-Cresol, with 2 uses in hair dyes. The FDA VCRP database in 2022 reports no change in uses for this ingredient; however, uses in hair dye products are now reported for 4-Amino-*m*-Cresol (35) and 5-Amino-6-Chloro-*o*-Cresol (27). Concentration of use were reported in the original report for all but 4-Chloro-2-Aminophenol. The highest concentration of use reported at the time was for 6-Amino-*m*-Cresol at 2.4%. A survey performed by the Council in 2022 had reported concentrations of use for 6-Amino-*m*-Cresol (0.69%), 4-Amino-*m*-Cresol (0.08% - 0.14%), and 5-Amino-6-Chloro-*o*-Cresol (0.007% - 0.24%): no concentrations of use were reported for the remaining ingredients.

A search of published literature studies dated 2002 forward discovered an IARC monograph (2020) for 4-Chloro-2-Aminophenol. The 2020 determination found inadequate evidence in humans for carcinogenicity, but sufficient evidence in experimental animals for carcinogenicity. 4-Chloro-2-Aminophenol is possibly carcinogenic to humans (Group 2B). No carcinogenicity data were identified in the original report for this ingredient.

Additionally, the European Commission (2004 SCCNFP opinion) determined that 4-Chloro-2-Aminophenol could not be considered safe for hair dying purposes due to the lack of an adequate safety dossier. This opinion also pertains to 6-Amino-*o*-Cresol. Opinions were separately written for 6-Amino-*m*-Cresol (2012 SCCS; "not safe for consumers"), 4-Amino-*m*-Cresol (2005 SCCP; "does not pose a risk to the health of the consumer"), and 5-Amino-6-Chloro-o-Cresol (2009 SCCS; "poses a risk to the health of the consumer"). These latter 3 opinions have toxicokinetic, dermal absorption, and toxicological studies that were not available at the time the original report was written, including positive genotoxicity data (6-Amino-*m*-Cresol, both in vitro and in vivo) and subchronic toxicity data with no NOAEL determined (6-Amino-*m*-Cresol). Further toxicokinetic, dermal absorption, and toxicological studies were also found for 4-Amino-*m*-Cresol in the ECHA database, including positive in vitro genotoxicity studies.

At the time the original report was written, there were no restrictions on the use of 4-Chloro-2-Aminophenol in cosmetics in Europe; however, European regulations regarding cosmetic ingredients now categorize 4-Chloro-2-Aminophenol in Annex II, the list of substances prohibited in cosmetic products in Europe. Annex II also lists 6-Amino-*m*-Cresol and 6-Amino-*o*-Cresol. Annex II lists 4-Amino-*m*-Cresol (limited to use in oxidative hair dyes and products intended for coloring eye lashes) and 5-Amino-6-Chloro-*o*-Cresol (concentration limited to 0.5% in non-oxidative hair dye products, use in oxidative hair dye products listed without a concentration limit),

No new data or opinions were found for 5-Amino-4-Chloro-*o*-Cresol.

Re-Review - Acid Orange 3 - History and New Data

(Christina Burnett – June 2022 meeting)

Ingredients (1)	Citation	Conclusion	Use - New Data	Results	Use - Historical Data	Results	Notes
Acid Orange 3 CAS# 6373-74-6	IJT 19(S1): 1-9, 2000	safe for use in hair dye formulations at concentrations ≤ 0.2%	frequency of use (2022) conc of use (2022)	1 no uses reported	frequency of use (1997) conc of use (1984)	4 up to 1% in 33/34 formulations, 1 formulation reported to be used at 10%-25%	The one current use of Acid Orange 3 is reported to be in a nail polish and enamel. Council survey indicated this ingredient is not in current use. During the writing of the original safety assessment, FDA had discontinued providing concentration of use data and the Council was not providing it to CIR at that time. The data in the original safety assessment are from data accessed from the FDA in 1984.

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Dinardo J and Draelos ZD. 2007. An animal model assessment of common dye-induced allergic contact dermatitis	Dermal sensitization – animal model comparison	Positive allergic reaction in a Buehler guinea pig sensitization test following induction with 10% Acid Orange 3 in propylene glycol and challenges at 2.5%, 5.0%, or 10%.	No dermal irritation/sensitization data in the original report; however, hair dyes in general may be sensitizing.
SCCNFP on 23 April 2004	Opinion on hair dyes without files submitted to the European Commission	The SCCNFP opined that Acid Orange 3 could not be considered safe for hair dying purposes due to the lack of an adequate safety dossier.	Previous safety assessment reported no restrictions in Europe.
COSING search 4/19/2022	Annex II, list of substances prohibited in cosmetic products in Europe	Acid Orange 3 (listed as entry #1280 under "Benzenesulfonic acid, 5-[(2,4-dinitrophenyl]amino]-2-(phenylamino)-, and its salts, when used as a substance in hair dye products"	Previous safety assessment reported no restriction in Europe

Search (from 1997 on)

PubMed

("acid orange 3") OR (228-921-5[EC/RN Number]) OR (6373-74-6[EC/RN Number])-1hit; relevant

ECHA

Entry for CAS # 6373-74-6 resulted in finding a dossier for "sodium 2-anilino-5-(2,4-dinitroanilino)benzenesulphonate". Toxicity data in the dossier are the same as those found in the original safety assessment.

Re-Review - *N,N*-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate - History and New Data

(Christina Burnett – June 2022 meeting)

Ingredients (1)	Citation	Conclusion	Use - New Data	Results	Use - Historical Data	Results	Notes
N,*N*-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate CAS# 54381-16-7; 57524-61-5; 58262-44-5	JACT 11(1):129-43, 1992	safe as a cosmetic ingredient in present practices of use and concentration	frequency of use (2022) conc of use (2022)	193 0.006%-1.3%	frequency of use (1984) conc of use (1984)	183 ≤ 5%	Uses have increased by 10 since the original report. In original report, 119 uses were reported as ≤ 0.1%, 55 uses reported between 0.1%-1%, and 9 uses reported between 1%-5%.

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Hgiwara A, Miyata E, Tamano S, et al. 1996. Non-carcinogenicity of 2,2'—(4-aminophenyl)imino-bisethanol sulfate in a long-term feeding study in Fischer 344 rats. Food Chem Toxicol. 34(6):537-546.	Carcinogenicity – dietary administration in groups of male and female rats for 104 wk; administered at 0, 300, 1000, or 3000 ppm	Authors concluded test material was not carcinogenic; mean body weights of both sexes in the 3000 ppm group were lower than the controls for the majority of the study; no treatment-related clinical signs or adverse effects observed in mortalities, feed consumption, or hematology; very slight but statistically significant increases in relative thyroid weights observed in he 3000 ppm group males, but no significant treatment-related increases in the incidence of non-neoplastic or neoplastic lesions.	Original report had negative data for hepatocarcinogencity.
SCCP on 20 June 2006, SCCP/0983/06, Opinion on N,N-Bis(2-Hydroxyethyl)-p-Phenylenediamine Sulfate	Dermal absorption	In peroxide developer mix: the overall mean recovery of the test material was 97.5%. Penetrated amount (receptor fluid + dermis/epidermis) after 48 h was 0.108 ± 0.064 µg/ cm^2, corresponding to 0.031% dermal absorption of the applied dose. In placebo developer mix: overall mean recovery of the test material was 102%. Penetrated amount after 48 h was 0.188 ± 0.242 µg/cm^2, corresponding to 0.054% dermal absorption of the applied dose. The maximum observed dermal absorption in the presence of developer mix (252 µg/cm^2) was used to calculate a margin of safety.	No dermal absorption data in the original report.
	Acute oral toxicity	LD_{50} calculated to be 107 mg/kg bw in male Tac N (SD) rats. Groups of 5 males received 50, 100, 200, or 400 mg/kg in 4% suspension in 1% methyl cellulose LD_{50} calculated to be 427 mg/kg bw in male Tac N (SD) rats. Groups of 5 males received 200, 400, 800, or 1600 mg/kg in 10% suspension in 1% methyl cellulose	LD_{50} in oral study was 246 mg/kg
	Short-term oral toxicity	NOAEL = 5 mg/kg bw/d. Test material tested at 0, 50, 100, 200, or 400 mg/kg bw/d in 0.2% w/v erythorbic acid in reverse osmosis water daily for 14 d via gavage in male and female rats. Due to toxicity and mortality after Day 2, the 50 and 100 mg/kg bw doses were reduced to 5 and 25 mg/kg bw, respectively. All animals given 200 or 400 mg/kg/d and 2 given 100 mg/kg/d died or were killed in a moribund condition. Surviving animals in the 100/25 dose group had thin appearance, mild tremors, reduced fecal output, rough hair coats, ocular fasciculation (females only) and swaying gait (females only). No clinical observations in control or 50/5 mg/kg d dose group.	In a 5 wk study in rats tested at up to 0.75% of the test material, no mortalities during treatment were reported. Hyperactivity reported in males in the 0.75% dose group. Adverse effects were observed in the kidneys and livers (kidney and liver to body weight changes, appearance, and organ weights). Some changes were attributed to diet rejection at the higher concentrations.
	Subchronic oral toxicity	NOAEL = 20 mg/kg/d. Test material tested at 0, 1, 4, or 20 mg/kg bw/d in 0.2% erythorbic acid in reverse osmosis water daily for 91 d via gavage in male and female rats. No adverse effects noted at any dose level during the testing period.	In a 13 wk, study in rats tested at up to 0.3% of the test material, no mortalities or signs of toxicity were observed. Only dose-related abnormality observed at necropsy was darkened thyroid glands in both males and females of the high dose group and in 1 male of the mid-dose group. High dose males also had significantly reduced pituitary gland weights when compared to the controls.

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
	DART	Maternal NOAEL = 5 mg/kg/d based on reductions in body weight and feed consumption; fetal NOAEL = 50 mg/kg/d. Test material tested at 0, 5, 20, or 50 mg/kg bw/d via oral gavage in rats on days 6-20 of gestation. The maternal NOAEL was used to calculate the margin of safety	In the original report, no embryonic or teratogenic effects observed in rat dams or fetuses that received up to 0.3% of the test material via oral gavage; or in rats that received up to 1% of the test material dermally. Oral administration of up to 0.3% of the test material did not produce a dominate lethal effect in rats.
	Genotoxicity – in vitro	Ames test at concentrations of 2.5 – 5000 µg/plate with and without metabolic activation: test material increased revertant count in *E.coli* strain WP2uvrA without metabolic activation. A positive result occurred in 1 out of 3 trials with *Salmonella* strain TA100 without metabolic activation Chromosome aberration test in Chinese hamster ovary cells at concentrations of 1.88 – 60 µg/ml without metabolic activation (up to 20 h incubation) and 50-700 µg/ml with metabolic activation (4 h incubation): test material induced aberrations with metabolic activation Mouse lymphoma (L5178Y *tk*+/-) mutation assay at concentrations 1.0-10 µg/ml without metabolic activation and 20-100 µg/ml with metabolic activation: negative for induction forward mutations with or without metabolic activation	No in vitro genotoxicity data in the original report.
	Genotoxicity – in vivo	Mouse bone marrow micronucleus test at concentrations of 15.625, 31.25, or 62.5 mg/kg administered in a single oral gavage: not genotoxic Rat liver UDS assay at concentrations of 100 and 200 mg/kg bw: negative	No in vivo genotoxicity data in the original report.
	Carcinogenicity – dermal	Dermal carcinogenicity studies in mice and rats: negative for carcinogenicity when tested up to 0.5%, with and without mixing with hydrogen peroxide	Results in tests with rats were negative for hepatocarcinogenicity in the original report. Test material was also not a promoter of hepatic neoplasms
	Dermal irritation – animal studies	Non-irritating in male and female New Zealand white rabbits after single application of 500 mg of aqueous slurry of test material was applied to shaved intact and abraded skin for 24 h	No dermal irritation data in the original report.
	Dermal sensitization – animal studies	Sensitizing in a guinea pig sensitization study of a 3% solution of the test material in Schultz Hamburg Vehicle II. 3 animals died during treatment period with respiratory conditions or diarrhea, positive challenge reactions noted in 5 of 7 surviving animals. Score were 1 for erythema except for a single score of 2 at 72 h post challenge. Moderate irritation observed during the initiation period. Test material was a strong sensitizer in 2 out of 3 local lymph node assays in CBA/Ca mice. The EC_3 value was 1.04% when tested in DMSO at 0.5%, 2.5%, or 5.0% in one test and all concentrations were positive when tested in DMSO at 0.25%, 0.5%, 1.0%, or 5.0%. In the third test, the SI values were just below 3 when tested in Acetone/Aqua/Olive Oil at 0.5%, 1.5%, or 2/8%.	Results in guinea pig tests were mixed and there were no LLNA data available.
	Ocular irritation – animal studies	Irritating in rabbit eyes (species not reported) after 100 mg of test material was instilled. Positive reactions in all rabbits. Non-irritating in rabbit eyes (species not reported) after 0.1 ml of test material in 1% w/v propylene glycol was instilled. No positive reactions	No ocular irritation data in the original report.
	Margin of safety (MOS) calculation	MOS was determined to be 1667 based on the maximum absorption through the skin of 0.252 µg/cm^2 and the NOAEL of 5 mg/kg.	No MOS calculation in the original report
	SCCP Conclusion	"…the SCCP is of the opinion that the use of *N*,*N*-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate itself as an oxidative hair dye substance at a maximum concentration of 2.5% in the finished cosmetic product (after mixing with hydrogen peroxide) does not pose a risk to the health of the consumer, apart from its sensitizing potential." *"N*,*N*-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate is a tertiary amine, and thus it is prone to nitrosation. It should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb."	No European standards reported in original safety assessment.

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
COSING search 4/20/2022	Annex 3, list of substances which cosmetic products must not contain except subjects to the restrictions laid down	Hair due substance in oxidative hair dye products; maximum concentration applied to hair (after mixing under oxidative conditions) must not exceed 2.5% (calculated as sulfate)	No European standards reported in original safety assessment.

Search (from 1990 on)

PubMed

((((N,N-Bis(2-Hydroxyethyl)-p-Phenylenediamine Sulfate) OR (54381-16-7[EC/RN Number])) OR (57524-61-5[EC/RN Number])) OR (58262-44-5[EC/RN Number])) OR (259-134-5[EC/RN Number])-2 hits; 1 relevant

ECHA

Entry for CAS # 54381-16-7 resulted in finding a dossier for "(p-ammoniophenyl)bis(2-hydroxyethyl)ammonium sulphate". Includes unpublished data not included in the original safety assessment; data was summarized in the 2006 SCCP Opinion.

Re-Review - 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Chloro-2-Aminophenol - History and New Data

(Christina Burnett – June 2022 meeting)

Ingredients (6)	Citation	Conclusion	Use - New Data	Results	Use - Historical Data	Results	Notes
6-Amino-*m*-Cresol CAS# 2835-98-5	IJT 23(S2): 1-22, 2004	The available data support the safety of 6-Amino-*m*-Cresol as used in oxidative and nonoxidative (semipermanent) hair dyes.	frequency of use (2022) conc of use (2022)	2 0.69%	frequency of use (1998) conc of use (1999)	2 2.4%	No change in # of uses, maximum concentration of use has decreased from 2.4% to 0.69%.
6-Amino-*o*-Cresol CAS# 17672-22-9		The available data support the safety of 6-Amino-*o*-Cresol for use in oxidative hair dyes, but are insufficient to support the safety in nonoxidative (semipermanent) hair dyes	frequency of use (2022) conc of use (2022)	None reported None reported	frequency of use (1998) conc of use (1999)	None reported 0.7%	No change in the # of uses, no reported concentration of use in 2022.
4-Amino-*m*-Cresol CAS# 2835-99-6		The available data support the safety of 4-Amino-*m*-Cresol as used in oxidative and nonoxidative (semipermanent) hair dyes.	frequency of use (2022) conc of use (2022)	35 0.08%-0.14%	frequency of use (1998) conc of use (1999)	None reported 0.3%	# of uses increased from none to 35, maximum concentration of use has decreased from 0.3% to 0.14%.
5-Amino-4-Chloro-*o*-Cresol CAS# 110102-86-8		The available data support the safety of 5-Amino-4-Chloro-*o*-Cresol as used in oxidative and nonoxidative (semipermanent) hair dyes.	frequency of use (2022) conc of use (2022)	None reported None reported	frequency of use (1998) conc of use (1994)	None reported up to 1% (after mixing with hydrogen peroxide)	No change in # of uses, no reported concentration of use in 2022.
5-Amino-6-Chloro-*o*-Cresol CAS# 84540-50-1		The available data support the safety of 5-Amino-6-Chloro-*o*-Cresol as used in oxidative and nonoxidative (semipermanent) hair dyes.	frequency of use (2022) conc of use (2022)	27 0.007%-0.24%	frequency of use (1998) conc of use (1996)	None reported 2%	# of uses increased from none to 27, maximum concentration of use has decreased from 2% to 0.24%.
4-Chloro-2-Aminophenol CAS# 95-85-2		The available data support the safety of 4-Chloro-2-Aminophenol for use in oxidative hair dyes, but are insufficient to support the safety in nonoxidative (semipermanent) hair dyes.	frequency of use (2022) conc of use (2022)	None reported None reported	frequency of use (1998) conc of use (1999)	None reported None reported	No change in # of use or concentration of use.

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
6-Amino-*m*-Cresol			
COSING search 4/23/2022	Annex II, list of substances prohibited in cosmetic products	Prohibited from use in the EU.	6-Amino-*m*-Cresol did not appear in Annex II when the safety assessment was originally reviewed.
SCCS on 26-27 June 2012, SCCS/1400/11, Opinion on 6-Amino-m-cresol (INCI) 2-Amino-5-methylphenol	ADME	[^{14}C]6-Amino-*m*-Cresol administered orally was well absorbed, readily distributed, extensively metabolized and excreted mainly via urine. There is weak analytical evidence that metabolism resulted in oxidized and *N*-acetylated derivatives. After dermal application, absorption was 5.1% (0.019 mg/cm^2) from excretion, cage-wash, carcass and unexposed skin, and 6.8% (0.026 mg/cm^2) when adding the residue in the exposed skin. Excretion took place mainly via urine but elimination was slower compared to oral administration. The bioavailability of 6-Amino-*m*-Cresol across the intestinal barrier was investigated in human intestinal epithelial (TC-7) cells in vitro. 6-Amino-*m*-Cresol (96 % recovery) revealed a P_{app} of 129.9 x 10-6 cm/sec and thus was classified to be of high permeability, indicating a complete absorption from the gastro-intestinal tract. As the absorption from the gastro-intestinal tract is likely to be permeability limited, the high permeability observed in this assay indicates a good absorption of 6-Amino-*m*-Cresol after oral administration. The metabolism of 6-Amino-*m*-Cresol is similar for human, rat and mouse primary hepatocytes. The test substance was extensively metabolized by sulfate and glucuronide conjugation. Although the human donors were phenotyped as rapid acetylators, no *N*-acetyl-2-amino-5-methylphenol could be detected. The metabolism of 6-Amino-*m*-Cresol from a typical oxidative hair dye formulation was investigated in viable human skin obtained from 3 female donors. The metabolite profiling results indicate that *N*-acetylation is the major route of metabolism of 6-Amino-*m*-Cresol in skin. *N*-acetyl-6-Amino-*m*-Cresol and two of its *O*-conjugates (sulfate ester and glucuronide) were found in receptor fluid and skin. The amount of *N*-acetylated metabolites was calculated to be 0.93 µg/cm^2, i.e at least 34% of the total amount of 6-Amino-*m*-Cresol that was found in the receptor fluid or in the skin (2.77 µg/cm^2) was present in the form of these *N*-acetylated metabolites. However, the acetylator status of the skin samples of the 3 donors regarding NAT1 (rapid or slow) is unknown. Thus, apart from other methodological restrictions, the evidence on the *N*-acetylation metabolic pathway in human skin is at best of semi-quantitative nature.	No ADME data in the original report.
	Dermal absorption	In vivo rat study, 0.58% of applied dose of 6-Amino-m-Cresol (15 mg/g) in a hair dye formulation and 14.25% of the applied dose of the test material dissolved in DMSO (150 mg/ml) were bioavailable. In vitro human skin, dermal absorption of an oxidative hair dye formulation containing 1.5% 6 Amino-m-Cresol (final concentration = 0.75%) was 2.77 µg/cm^2 (0.34%).	No dermal absorption data in the original report.
	Acute oral toxicity	LD_{50} for male Wistar rats = 1375 mg/kg (tested at up to 1750 mg/kg) LD_{50} for female Wistar rats = 1225 mg/kg (tested at up to 1500 mg/kg) LD_{50} for male CF1 mice = 1020 mg/kg (tested at up to 1500 mg/kg) LD_{50} for female CF1 mice = 1225 mg/kg (tested at up to 2000 mg/kg) LD_{50} for female CBL mice = 750 mg/kg (tested at up to 1250 mg/kg)	LD_{50} reported to be 1500 mg/kg in male CD-1 mice in original safety assessment.
	Subchronic oral toxicity	No NOAEL identified after 800 mg/kg bw/d (reduced to 500 after wk 6) test material (10% suspension in 5% gum Arabic was administered to male and female rats for 90 d via gavage. Feed consumption body weight and body weight gain were significantly reduced in both sexes; relative and absolute liver, kidney, and spleen weights were increased; no macroscopic or histopathological effects detected.	No subchronic oral toxicity studies for 6-Amino-*m*-Cresol in the original safety assessment.

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
	Genotoxicity – in vitro	Ames test at concentrations up to 5000 µg/plate in *Salmonella typhimurium* strains TA 98, TA100, TA 1535, TA 1537, and TA 102, with and without metabolic activation: mutagenic in TA 100 with and without metabolic activation. Mouse lymphoma (L5178Y *tk*+/-) mutation assay at concentrations 0.1-160 µg/ml without metabolic activation and 0.5-100 µg/ml with metabolic activation: a concentration dependent and biologically relevant increase in mutant frequency observed with metabolic activation, an increased occurrence of small colonies was found indicating a mutagenic or clastogenic effect. Micronucleus test in human lymphocytes at concentrations 8.6-26.8 µg/ml without metabolic activation and 25.0-67.7 µg/ml with metabolic activation: 6-Amino-*m*-Cresol induced an increase in lymphocytes with micronuclei without metabolic activation Comet assay in V79 cells at concentrations 25-1232 µg/ml without metabolic activation and 308-1232 µg/ml with metabolic activation: a concentration-dependent and biologically relevant increase in the amount of DNA in the tail was observed with and without metabolic activation.	In original safety assessment, 6-Amino-*m*-Cresol was slightly mutagenic towards *S. typhimurium* TA 100, with and without metabolic activation, but not mutagenic to other strains. Genotoxicity studies in yeast cells, mouse lymphoma L5178Y cells, and human peripheral lymphocytes were negative.
	Genotoxicity – in vivo	Mammalian erythrocytes micronucleus test in rats at concentrations 0, 100, 200, or 400 mg/kg bw in 2.5% hydroxypropylcellulose: 6-Amino-*m*-Cresol induced an increase in the number of bone marrow cells with micronuclei and was considered to be genotoxic.	In the original safety assessment, 6-Amino-*m*-Cresol was not genotoxic in 3 different micronucleus studies, a chromosome aberration study, or an unscheduled DNA synthesis assay.
	Dermal irritation – animal studies	Dermal irritation study in albino guinea pigs at 1% in water, thickened with methylcellulose, applied 3x daily for 2 consecutive days: negligible erythema on 1st day, not recognizable on 2nd day, no edema or crusts	No animal dermal irritation data in the original safety assessment.
	Dermal sensitization – animal studies	Magnusson-Kligman method in guinea pigs at 3% in water, thickened with 0.5% tylose: not sensitizing LLNA, test concentrations were 0.5%, 1.5%, 5%, or 10% in DMSO and 0.5%, 1.5%, 3%, or 5% in acetone: water (1:1) mixed with olive oil (3:1): strong skin sensitizer	No animal dermal sensitization data in the original safety assessment.
	Ocular irritation studies	Ocular irritation study in guinea pigs, 1% aqueous solution, instilled into 1 eye: not irritating after 24 h observation	No ocular irritation data in the original safety assessment.
	SCCS Conclusion	"…the SCCS considers that [6-Amino-*m*-Cresol] is not safe for consumers, when used in oxidative hair dye formulations with a concentration on the scallop of maximum 1.5% taking into account the scientific data provided. The dimer of [6-Amino-*m*-Cresol] is probably formed under the oxidative hair de conditions and was found to be absorbed by human skin in vitro. The dimer was also found in high concentrations in a study with human hepatocytes when the concentration of the substrate [6-Amino-*m*-Cresol] was high. The effects of the dimer require further elucidation." Findings based on genotoxicity potential of the test material and its metabolite and the lack of adequate data that the test material is completely converted to non-toxic metabolites in the skin in vivo.	Previous safety assessment reported no restrictions in Europe.
6-Amino-*o*-Cresol			
COSING search 4/23/2022	Annex II, list of substances prohibited in cosmetic products	Prohibited from use in the EU.	6-Amino-*o*-Cresol did not appear in Annex II when the safety assessment was originally reviewed.
SCCNFP on 23 April 2004	Opinion on hair dyes without files submitted to the European Commission	The SCCNFP opined that 6-Amino-*o*-Cresol could not be considered safe for hair dying purposes due to the lack of an adequate safety dossier.	Previous safety assessment reported no restrictions in Europe.
4-Amino-*m*-Cresol			
COSING search 4/23/2022	Annex III, list of substances which cosmetic products must not contain	Limited to use in oxidative hair dyes and products intended for coloring eye lashes.	Previous safety assessment reported no restrictions in Europe.

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
SCCP on 20 September 2005, SCCP/0898/05, Opinion on 4-Amino-m-cresol	Toxicokinetics	A mean permeability in human intestinal epithelial (TC-7) cells of 59 $x10^{-6}$ cm/sec was obtained, which classifies 4-Amino-*m*-Cresol to be of high permeability. As the absorption from the gastro-intestinal tract is likely to be permeability limited, the high permeability observed in this assay indicates a good absorption of 4-Amino-*m*-Cresol after oral administration.	No toxicokinetics data in the original report.
	Dermal absorption	In vivo rat study, a mean absorption rate of 41.4 µg/cm² was found for 4-Amino-*m*-Cresol when applied in a commercial formulation in the presence of peroxide under typical use conditions In vitro pig skin study, 8.3-66.5 µg/cm² (0.6%-4.4%) 4-Amino-*m*-Cresol was bioavailable when tested as part of a hair dye formulation at 1.5%.	No dermal absorption data in the original report.
	Acute oral toxicity	LD_{50} for female Wistar rats = 1010 mg/kg (tested at up to 1200 mg/kg) LD_{50} for male Wistar rats = 870 mg/kg (tested at up to 1100 mg/kg) LD_{50} for female CF1 mice = 908 mg/kg (tested at up to 1000 mg/kg)	LD_{50} reported to be 1000 mg/kg in male CD-1 mice in original safety assessment.
	Genotoxicity – in vitro	Ames test at concentrations up to 5000 µg/plate in *S. typhimurium* strains TA98, TA100, TA 102, TA 1535, and TA 1537, with and without metabolic activation: not mutagenic Mouse lymphoma (L5178Y *tk+/-*) mutation assay at concentrations 0.048-6.25 µg/ml without metabolic activation and 0.391-47.5 µg/ml with metabolic activation: not mutagenic	In original safety assessment, 4-Amino-*m*-Cresol was not mutagenic in an Ames test, with or without metabolic activation. The test material did not induce UDS in rat hepatocytes.
	Genotoxicity – in vivo	Mammalian micronucleus test in mice at concentrations 20, 100, or 200 mg/bw via single i.p. injection: test material did not induce chromosome aberrations or damage to the mitotic apparatus	In original safety assessment, micronucleus assays, a sister chromatid exchange assay, and unscheduled DNA synthesis studies of 4-Amino-m-Cresol were negative for genotoxicity.
	Dermal irritation – animal studies	Dermal irritation study in albino Pirbright guinea pigs at 3% dilution in 0.5% tylose, applied daily for 5 consecutive days: not irritating	No dermal irritation data in the original safety assessment.
	Dermal sensitization – animal studies	Guinea pig maximization study with 3% 4-Amino-m-Cresol in distilled water and in Freund's complete adjuvant/arachidis oil for intradermal induction, 3% test material in white Vaseline (occluded, pretreatment with 10% sodium lauryl sulfate in white Vaseline) for dermal induction, 1%, 2%, and 3% test material in distilled water (occluded) for challenge: not sensitizing LLNA, test concentrations were 0.5%, 1.5%, 5%, or 10% in DMSO and 0.5%, 1.5%, 3%, or 5% in aqua/acetone (1:1) mixed with olive oil (4:1): moderate skin sensitizer	No dermal sensitization data in the original safety assessment.
	Ocular irritation studies	Ocular irritation study in guinea pigs, 1.5% dilution in 50% propylene glycol, instilled into 1 eye: minimally irritating	No ocular irritation data in the original safety assessment.
	Margin of safety (MOS)	The SCCP calculated a MOS of 124 based on the maximum absorption of 41.4 µg/cm² and a NOAEL of 60 mg/kg (from an oral rat subchronic study that had been summarized in the original safety assessment).	No margin of safety was calculated in the original safety assessment.
	SCCP Conclusion	"The SCCP is of the opinion that the use of 4-Amino-*m*-Cresol itself as an oxidative hair dye at a maximum concentration of 1.5% in the finished cosmetic product (after mixing with hydrogen peroxide) does not pose a risk to the health of the consumer, apart from its sensitizing potential."	Previous safety assessment reported no restrictions in Europe.
ECHA data base entry for "4-amino-m-cresol" accessed 4/26/2022	Toxicokinetics	4-Amino-*m*-Cresol was considered as low bioaccumulable potential substance by oral route and dermal route or intravenously according to the three in vivo key studies. The excretion rate (mainly by the urine) after exposure was calculated at 92% of bioavailable substance in this route. In oral route, the absorption rate was defined as 105% (oral gavage).	No toxicokinetics data in the original report.
	Dermal absorption	According to the in vitro key study which used the test item diluted at 2%, dermal absorption was determined at 0.471% using pig skin samples or 0.26% when human skin samples were used.	No dermal absorption data in the original report.
	Genotoxicity – in vitro	Micronucleus test in human lymphocytes at concentrations of 5-35 µg/ml without metabolic activation and 50-150 µg/ml with metabolic activation, all with pre-treatment with mitogen: 4-Amino-*m*-Cresol induced micronuclei with and without metabolic activation Chromosomal aberration test in human peripheral blood lymphocytes at concentrations of 1-20 µg/ml without metabolic activation and 39.1-156.3 µg/ml with metabolic activation: induced chromosomal aberrations with and without metabolic activation	In original safety assessment, 4-Amino-*m*-Cresol was not mutagenic in an Ames test, with or without metabolic activation. The test material did not induce UDS in rat hepatocytes.

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
	Dermal irritation studies – in vitro	EpiSkin™ reconstructed human derived epidermis in vitro assay with 4-Amino-*m*-Cresol tested neat: non-irritating EipDerm™ reconstructed human epidermis in vitro assay with test material tested neat: non-corrosive	No dermal irritation data in the original safety assessment.
	Ocular irritation studies	Isolated chicken eye in vitro tests with test material tested neat and as a 1.5% dilution with 50% propylene glycol: not irritating	No ocular irritation data in the original safety assessment.
5-Amino-4-Chloro-*o*-Cresol			
		no pertinent new data	
5-Amino-6-Chloro-*o*-Cresol			
COSING search 4/23/2022	Annex III, list of substances which cosmetic products must not contain	Concentration limited to 0.5% in non-oxidative hair dye products. Use in oxidative hair dye products listed without a concentration limit.	Previous safety assessment reported no restrictions in Europe.
SCCS on 8 December 2009, SCCS/1225/09, Opinion on 5-Amino-6-chloro-o-cresol			
	Dermal absorption	In vitro pig skin where 5-Amino-6-Chloro-*o*-Cresol was tested in hair dye formulations at a final concentration of 2.1%, the amounts considered absorbed were 30.21 µg/cm² with peroxide and 53.8 µg/cm² without peroxide.	No dermal absorption data in the original report.
	Subchronic oral toxicity	NOAEL = 100 mg/kg/d (SCCS says this should be a LOAEL) when tested at 0, 100, 300, or 600 m/kg bw in propylene glycol for 90 d via oral gavage (28 d recovery); adverse effects included centrilobular hypertrophy of the liver observed at all dose levels in a dose-dependent manner that persisted through the recovery period; high dose male group also had cortical tubular basophilia in the kidneys, limiting ridge hyperplasia of the forestomach with squamous hyperplasia of the main stomach; increased relative and absolute liver weights were observed in the high dose; reduced mean corpuscular Hb concentration in all dose groups of female rats	NOAEL = 50 mg/kg when tested up to 50 mg/kg
	Genotoxicity – in vitro	Ames test at concentrations up to 5000 µg/plate in *S. typhimurium* strains TA 98, TA100, TA 102, TA 1535, TA 1537, with and without metabolic activation: not mutagenic Mouse lymphoma (L5178Y *tk+/-*) mutation assay at concentrations 25-1000 µg/ml without metabolic activation and 1.56-18.9 µg/ml with metabolic activation: 5-Amino-6-Chloro-o-Cresol did not induce gene mutations	In original safety assessment, 5-Amino-6-Chloro-*o*-Cresol (HCL) was mutagenic with metabolic activation, but was not genotoxic in studies with V79 Chinese hamster lung cells or in an unscheduled DNA synthesis study.
	Genotoxicity – in vivo	Mammalian erythrocytes micronucleus test in rats at concentrations 0, 100, 200, or 400 mg/kg bw in 30% DMSO: : 5-Amino-6-Chloro-o-Cresol did not induce micronuclei	Similar results in the original safety assessment.
	Dermal irritation – animal studies	Dermal irritation study in New Zealand White rabbits, 0.5 g test material moistened with 0.7 ml Milli-U water and applied to intact shaved skin for 4 h under semi-occlusive patch: not irritating or corrosive	5-Amino-6-Chloro-*o*-Cresol was negative for dermal toxicity in the original safety assessment.
	Dermal sensitization – animal studies	LLNA, test concentrations were 5%, 25%, or 50% in ethanol: water (7:3): non-sensitizing	Not sensitizing in a guinea pig maximization test when induced at 5% and challenged at 25%.
	Ocular irritation studies	Ocular irritation study in New Zealand White rabbits, ~0.1 ml undiluted test material instilled into 1 eye: irritating	Similar results in original assessment.
	Margin of safety (MOS)	The SCCS calculated a MOS of 51 for non-oxidative conditions and 68 for oxidative conditions. For the non-oxidative, the calculation was based on the absorption of 66.6 µg/cm² and a LOAEL of 100 mg/kg (adjusted by a factor of 3 to 33, from the oral subchronic study summarized above). For the oxidative, the calculation was based on the absorption of 49.77 µg/cm² and the same LOAEL.	No margin of safety was calculated in the original safety assessment.
	SCCS Conclusion	"Because of the low margin of safety for the use in both oxidative and non-oxidative hair dye formulations, the SCCS is of the opinion that the use of 5-amino-6-chloro-o-cresol as a hair dye ingredient up to a final on-head concentration of 2.0% under oxidative and non-oxidative conditions poses a risk to the health of the consumer.	Previous safety assessment reported no restrictions in Europe.
4-Chloro-2-Aminophenol			
COSING search 4/23/2022	Annex II, list of substances prohibited in cosmetic products	Prohibited for use in the EU.	4-Chloro-2-Aminophenol did not appear in Annex II when the safety assessment was originally reviewed.

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
SCCNFP on 23 April 2004	Opinion on hair dyes without files submitted to the European Commission	The SCCNFP opined that 4-Chloro-2-Aminophenol could not be considered safe for hair dying purposes due to the lack of an adequate safety dossier.	Previous safety assessment reported no restrictions in Europe.
IARC Monograph 2020	Genotoxicity – in vitro	Ames tests (concentrations not reported): positive with metabolic activation in *Salmonella typhimurium* strains TA 100 and TA 1537; negative without metabolic activation in any strain tested Chromosomal aberration assay in Chinese hamster lung cells (concentrations not reported): positive with and without metabolic activation	4-Chloro-2-Aminophenol was weakly mutagenic in an Ames test using concentration of 10 - 1500 µg/plate, with metabolic activation, in strain TA 1535.
	Carcinogenicity (Full data sets in reports by the Japan Bioassay Research Center, 2008)	Oral mice studies at 0, 512, 1280, or 3200 ppm for 2 yr: test material induced a significant positive trend in the incidence (males and females) and a significant increase in the incidence of squamous cell papillomas (males) of the forestomach. Oral rat studies at 0, 1280, 3200, or 8000 ppm for 2 yr: test material induced a significant positive trend in the incidence (males and females) and a significant increase in the incidence of squamous cell papilloma (males and females), squamous cell carcinoma (males), or squamous cell papilloma or carcinoma (combined) of the forestomach. In males, the test material also induced a significant positive trend in the incidence and a significant increase in the incidence of transitional cell carcinoma of the urinary bladder.	No carcinogenicity data on 4-Chloro-2-Aminophenol was in the original safety assessment.
	Findings	IARC determined "there is inadequate evidence in humans for the carcinogenicity of [4-chloro-2-aminophenol]. There is sufficient evidence in experimental animals for the carcinogenicity of [4-chloro-2-aminophenol]. [4-Chloro-2-aminophenol] is possibly carcinogenic to humans (Group 2B)."	No carcinogenicity data on 4-Chloro-2-Aminophenol was in the original safety assessment.

<u>Search (from 2002 on)</u>
PubMed
6-Amino-m-Cresol
(("6-Amino-m-Cresol") OR (2835-98-5[EC/RN Number])) - 10 hits; 0 relevant
6-Amino-o-Cresol
(("6-Amino-o-Cresol") OR (17672-22-9[EC/RN Number])) – 0 hits
4-Amino-m-Cresol
(("4-Amino-m-Cresol") OR (2835-99-6[EC/RN Number])) – 6 hits; 0 relevant
5-Amino-4-Chloro-o-Cresol
(("5-Amino-4-Chloro-o-Cresol") OR (110102-86-8[EC/RN Number])) – 1 hit; 0 relevant
5-Amino-6-Chloro-o-Cresol
(("5-Amino-6-Chloro-o-Cresol") OR (84540-50-1[EC/RN Number])) – 1 hit; 0 relevant
4-Chloro-2-Aminophenol
(("4-Chloro-2-Aminophenol") OR (95-85-2[EC/RN Number])) – 16 hits; 0 relevant

ECHA
6-Amino-m-Cresol
No dossier for CAS# 2835-98-5.
6-Amino-o-Cresol
No dossier for CAS# 17672-22-9.
4-Amino-m-Cresol
Entry for CAS# 2835-99-6 resulted in finding a dossier for "4-amino-m-cresol". Data not summarized in the SCCP are summarized above.

5-Amino-4-Chloro-o-Cresol
No entries.
5-Amino-6-Chloro-o-Cresol
No entries.
4-Chloro-2-Aminophenol
Entry for CAS # 95-85-2 resulted in finding a dossier for "2-amino-4-chlorophenol". Limited data included an oral LD_{50} of 690 in rats and a rat oral carcinogenicity study outlined in IARC.

Current and historical frequency and concentration of use according to duration and exposure

	Acid Orange 3				***N*,*N*-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate**			
	# of Uses		**Max Conc of Use (%)**		**# of Uses**		**Max Conc of Use (%)**	
	2022[1]	**1997[2]**	**2022[3]**	**1984[2]**	**2022[1]**	**1984[4]**	**2022[5]**	**1984[4]**
Totals*	**1**	**4**	**NR**	**up to 1, 10-25ǂ**	**193**	**183**	**0.006-1.3**	**< 5‡**
Duration of Use								
Leave-On	*1*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Rinse-Off	*NR*	*4*	*NR*	*up to 1, 10-25ǂ*	*193*	*183*	*0.006-1.3*	*< 5‡*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	NR	NR	NR	NR	NR	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Hair-Coloring	NR	4	NR	up to 1, 10-25ǂ	193	183	0.006-1.3	< 5‡
Nail	1	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	6-Amino-*m*-Cresol				**6-Amino-*o*-Cresol**			
	# of Uses		**Max Conc of Use (%)**		**# of Uses**		**Max Conc of Use (%)**	
	2022[1]	**1998[6]**	**2022[7]**	**1999[6]**	**2022[1]**	**1998[6]**	**2022[7]**	**1999[6]**
Totals*	**2**	**2**	**0.69**	**2.4**	**NR**	**NR**	**NR**	**0.7**
Duration of Use								
Leave-On	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Rinse-Off	*2*	*2*	*0.69*	*2.4*	*NR*	*NR*	*NR*	*0.7*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	NR	NR	NR	NR	NR	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Hair-Coloring	2	2	0.69	2.4	NR	NR	NR	0.7
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR
	4-Amino-*m*-Cresol				**5-Amino-4-Chloro-*o*-Cresol**			
	# of Uses		**Max Conc of Use (%)**		**# of Uses**		**Max Conc of Use (%)**	
	2022[1]	**1998[6]**	**2022[7]**	**1999[6]**	**2022[1]**	**1998[6]**	**2022[7]**	**1994[6]**
Totals*	**35**	**NR**	**0.08-0.14**	**0.3**	**NR**	**NR**	**NR**	**up to 1ǂǂ**
Duration of Use								
Leave-On	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Rinse-Off	*35*	*NR*	*0.08-0.14*	*0.3*	*NR*	*NR*	*NR*	*up to 1ǂǂ*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	NR	NR	NR	NR	NR	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Hair-Coloring	35	NR	0.08-0.14	0.3	NR	NR	NR	up to 1ǂǂ
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

Current and historical frequency and concentration of use according to duration and exposure

	5-Amino-6-Chloro-*o*-Cresol				4-Chloro-2-Aminophenol			
	# of Uses		Max Conc of Use (%)		# of Uses		Max Conc of Use (%)	
	2022[1]	1998[6]	2022[7]	1996[6]	2022[1]	1998[6]	2022[7]	1999[6]
Totals*	**27**	**NR**	**0.007-0.24**	**2**	**NR**	**NR**	**NR**	**NR**
Duration of Use								
Leave-On	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Rinse-Off	*27*	*NR*	*0.007-0.24*	*2*	*NR*	*NR*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type								
Eye Area	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR	NR	NR	NR	NR
Dermal Contact	NR	NR	NR	NR	NR	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	NR	NR	NR	NR
Hair-Coloring	27	NR	0.007-0.24	2	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR	NR	NR

*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
ǂ The concentration of use data were presented in range groups at the time this report was written. For Acid Orange 3, the concentration was up to 1% in 33/34 formulations and 1 formulation reported that it was used between 10%-25%.
‡ The concentration of use data were presented in range groups at the time this report was written. For *N*,*N*-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate, 119 uses were reported at concentrations < 0.1%, 55 uses were reported between 0.1%-1%, and 9 uses were reported between 1%-5%.
ǂǂ After mixing with peroxide.
NR – not reported

1. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients. College Park, MD. 2022. (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2022; received January 11, 2022.)

2. Andersen FA (ed.). Final report on the safety assessment of Acid Orange 3. *Int J Toxicol.* 2000;19(Suppl 1):1-9.

3. Personal Care Products Council. 2022. Concentration of Use by FDA Product Category: Acid Orange 3. Unpublished data submitted by the Personal Care Products Council on January 12, 2022.

4. Elder RL (ed.). Final report on the safety assessment of *N*,*N*-Bis(Hydroxyethyl)-*p*-Phenylenediamine Sulfate. *J Am Coll Toxicol.* 1992;11(1):129-143.

5. Personal Care Products Council. 2022. Concentration of Use by FDA Product Category - N,N-Bis(2-Hydroxyethyl)-p-Phenylenediamine Sulfate. Unpublished data submitted by the Personal Care Products Council on January 10, 2022.

6. Andersen FA (ed.). Final report on the safety assessment of 6-Amino-m-Cresol, 6-Amino-o-Cresol, 4-Amino-m-Cresol, 5-Amino-4-Chloro-o-Cresol, 5-Amino-6-Chloro-o-Cresol, and 4-Chloro-2-Aminophenol. *Int J Toxicol.* 2004;23(Suppl. 2):1-22.

7. Personal Care Products Council. Concentration of Use by FDA Product Category: Amino Cresol Ingredients. 2022.

Final Report on the Safety Assessment of Acid Orange 3[1]

Acid Orange 3 is a nitro color used as a hair colorant. Short-term animal studies showed no toxicity at oral exposures less than 1.5 g/kg in rats and 1.0 g/kg in mice. Dermal exposure to a hair dye formulation containing 0.2% Acid Orange 3 did not cause adverse effects. Likewise, this level of dermal exposure was not associated with reproductive or developmental toxicity. Acid Orange 3 was mutagenic in the Ames test system and in one mammalian cell transformation system. The results of a dermal carcinogenicity study in mice exposed to a hair dye formulation containing 0.2% Acid Orange 3 were negative. Oral carcinogenicity studies in rats and mice did yield clear evidence of carcinogenic activity in female rats, but not in male rats or in male and female mice. Although there are no data on the irritation and sensitization potential of this ingredient, hair dyes containing Acid Orange 3 can be expected to carry the caution mandated by the Food and Drug Administration (FDA) that alerts users to the need to perform patch testing *on their own skin to determine whether the product causes skin* irritation. Following this admonition, individuals who would have an irritation/sensitization reaction can avoid significant exposure. Accordingly, the Expert Panel concluded that Acid Orange 3 is safe for use in hair dye formulations at concentrations less than or equal to 0.2%.

INTRODUCTION

Acid Orange 3 is a nitro color that functions as a hair colorant in hair dyes and colors (Wenninger, Canterbery, and McEwen 2000).

CHEMISTRY

Definition and Structure

Acid Orange 3 (CAS No. 6373-74-6) conforms to the formula shown in Figure 1 (Wenninger, Canterbery, and McEwen 2000). Acid Orange 3 is also known as C.I. 10385; 5-[(2,4-Dinitrophenol)amino]-2-(Phenylamino)Benzenesulfonic Acid, Monosodium Salt (National Toxicology Program [NTP] 1988; International Agency for Research on Cancer [IARC] 1993; Wenninger, Canterbery, and McEwen 2000); Benzenesulfonic Acid, 5-[(2,4-Dinitrophenyl)Amino]-2-(Phenylamino), Monosodium Salt; Amido Yellow EA (Wenninger, Canterbery, and McEwen 2000); C.I. Acid Orange 3; 2-Anilino-5-(2,4-Dinitroanilino)Benzenesulfonic Acid, Monosodium Salt (NTP 1988; IARC 1993); and *Sodium 4-(2,4-Dinitroanilino)Diphenylamine-2-Sulfonate* (IARC 1993).

Physical and Chemical Properties

Acid Orange 3 occurs as dark orange-brown microcrystals (NTP 1988). It has a molecular weight of 452.39 Da and is very soluble in water and ethanol (IARC 1993).

Manufacture and Production

Acid Orange 3 is prepared by the condensation of 1-chloro-2,4-dinitrobenzene with 5-amino-2-anilinobenzenesulfonic acid (Society of Dyers and Colourists 1971).

Analytical Methods

Acid Orange 3 has been identified by ultraviolet (UV) and nuclear magnetic resonance spectroscopy (NTP 1988) and analyzed by fast atom bombardment mass spectrometry (Ventura et al. 1989).

Impurities

Initial analysis of one batch of Acid Orange 3 by UV spectroscopy reported that it contained only 67% Acid Orange 3 (NTP 1988). After purification, analysis using UV spectroscopy and high performance liquid chromatography (HPLC) determined purity of 94.3% and 88.7%, respectively; gas chromatography and flame ionization established an acetone content of 2.7%. Analysis of a second batch by UV spectroscopy and HPLC established purity of 89.1% and 89.0%, respectively. UV analysis of this batch detected one impurity >1% at 280 nm; this impurity was not identified. HPLC did not detect any impurities (other than water) >1%.

USE

Cosmetic

Acid Orange 3 is reported to function as a hair colorant in hair dyes and colors (Wenninger, Canterbery, and McEwen 2000). The product formulation data submitted to the Food and Drug Administration (FDA) in 1997 reported that Acid Orange 3 was used in four cosmetic formulations (FDA 1997) (Table 1).

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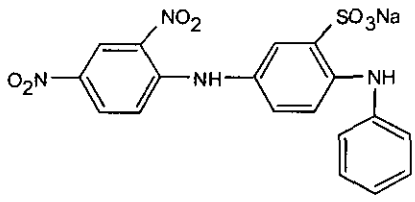

FIGURE 1
Chemical structure of Acid Orange 3.

Concentration of use values are no longer reported to the FDA by the cosmetic industry (FDA 1992). The product formulation data submitted to the FDA in 1984 stated that Acid Orange 3 was used in 34 hair dye/color formulations that required caution statements; one use was in the concentration range 10% to 25%; the remaining uses were at concentrations of ≤1% (FDA 1984) (Table 2).

Hair coloring formulations are applied to or may come in contact with hair, skin (particularly at the scalp), eyes, and nails. Individuals dyeing their hair may use such formulations once every few weeks, whereas hairdressers may come in contact with products containing these ingredients several times a day. Under normal conditions of use, skin contact with hair dye is restricted to 30 minutes.

Hair dyes containing Acid Orange 3, 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 of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The following caution statement should be displayed conspicuously on the labels of coal tar hair dyes:

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

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

> The Cosmetic Ingredient Review (CIR) Expert Panel has reviewed the cosmetic industry's current coal tar hair dye product labeling, which recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 24 h 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 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). These procedures state that the test material should be applied at an acceptable concentration to the patient, covered with an appropriate occlusive patch, and evaluated for sensitization 48 and 72 h after application. The CIR Expert Panel has cited the results of studies conducted by both the North American Contact Dermatitis Group and the International Contact Dermatitis Group in its safety evaluation reports on cosmetic ingredients (Elder 1985).
>
> During the August 26-27, 1991 public meeting of the CIR Expert Panel, all members agreed that the cosmetic industry should change its recommendation for the evaluation of the open patch test from 24 h to 48 h after application of the test material.
>
> The industry was advised of this recommendation and asked to provide any compelling reasons why this recommendation should not be made by the Expert Panel and adopted by the cosmetic industry. No opposition to this recommendation was received. At the February 11, 1992 public meeting of the CIR Expert Panel, this policy statement was adopted.

TABLE 1
Product formulation data (FDA 1997)

Product category	Total no. formulations in category	Total no. containing Acid Orange 3
Hair dyes and colors	1478	4
Total uses of Acid Orange 3 in 1997		**4**

TABLE 2
Concentration of use of Acid Orange 3 in cosmetic formulations (FDA 1984)

Product category	10–25%	0.1–1%	0–0.1%	Total uses in product category
Hair dyes/colors (requiring caution statements)	1	16	17	34
Total of uses of Acid Orange 3 in 1984				**34**

International

Acid Orange 3 does not appear in Annex II (list of substances that must not form part of the composition of cosmetic products), III (list of substances that cosmetic products must not contain except subject to the restrictions and conditions laid down), or IV (list of coloring agents allowed for use in cosmetic products) of the *Cosmetics Directive of the European Union* (European Economic Community 1995). Acid Orange 3 is not included in *Japan's Ministry of Health and Welfare Ordinance No. 30/1966*, and it is not published in the *Japanese Standards of Quasi-Drugs*, indicating that it is not an approved cosmetic colorant (Rempe and Santucci 1997).

Noncosmetic

Acid Orange 3 is used to dye textiles (IARC 1993).

GENERAL BIOLOGY

Cytotoxicity

Acid Orange 3 was cytotoxic to BALB/c-3T3 cells in tissue culture (Matthews, Spalding, and Tennant 1993). On average, the concentration that allowed a 50% survival was 0.102 mM.

ANIMAL TOXICOLOGY

Acute Toxicity

Published data on the acute toxicity of Acid Orange 3 were not found.

Short-Term Oral Toxicity

Groups of five male and five female F344/N rats were given 94, 187, 375, 750, or 1500 mg/kg Acid Orange 3 in corn oil by gavage for 14 days (NTP 1988). A control group was given vehicle only. All animals were observed twice daily for signs of toxicity. Body weights were determined on days 1, 7, and 15. All animals were necropsied.

One female of the 1500 mg/kg dose group died on day 16; all other animals survived to study termination. Final mean body weights and mean body weight gains were not "significantly affected" by oral administration of Acid Orange 3. Orange urine or extremities were observed for one female of the 94 mg/kg dose group, two females of the 187 mg/kg dose group, three males and four females of the 375 mg/kg dose group, and all animals of the 750 and 1500 mg/kg dose groups. Compound-related lesions were not seen at necropsy.

A study using B6C3F$_1$ mice, five per sex per group, was performed according to the same procedures as above (NTP 1988). The dose groups were given 62, 125, 250, 500, or 1000 mg/kg Acid Orange 3. All animals survived to study termination. Males of the 500 and 1000 mg/kg dose groups lost weight initially and had overall decreased body weight gains compared to the control group; this was attributed to a malfunctioning of the water system during week 1. Animals of all dose groups had orange urine and all but two mice of the 1000 mg/kg dose group were inactive. Compound-related lesions were not found at necropsy.

Subchronic Oral and Dermal Toxicity

F344/N rats, 10 per sex per group, were given 94, 187, 375, 750, or 1500 mg/kg Acid Orange 3 in corn oil by gavage 5 days per week for 13 weeks (NTP 1988). A control group was given vehicle only. All animals were observed twice daily. Body weights were determined at study initiation and then weekly. Microscopic examination was performed on tissues from all control and high-dose animals (adrenal glands, brain, colon, esophagus, femur including marrow, heart, kidneys, liver, lungs and bronchi, mandibular and mesenteric lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes/seminal vesicles or ovaries/uterus, salivary glands, skin, small intestine, spleen, stomach, thigh muscle, thymus, thyroid gland, trachea, and urinary bladder).

Five females of the 1500 mg/kg dose group died during weeks 1, 7, and 8 of the study; all other animals survived to study termination. Final mean body weights of males and females of the 1500 mg/kg dose group were decreased by 8% and 5% compared to control values, respectively. The haircoats of males of the 750 and 1500 mg/kg dose groups and all dosed females were discolored yellow. Of animals of the 1500 mg/kg dose group, nephrosis was observed in nine males and two females, suppurative inflammation of the kidney was observed in three females, and necrosis of the renal papilla was observed in two females. Of the females of the 1500 mg/kg dose group that survived until study termination, all five had acidophilic cytoplasmic inclusion bodies or granules in the transitional epithelium of the urinary bladder and two of the five had hyperplasia of the transitional epithelium of the urinary bladder.

A study using B6C3F$_1$ mice, 10 per sex per group, was performed according to the same procedures as above (NTP 1988). The dose groups were given 31, 62, 125, 250, or 500 mg/kg Acid Orange 3. No compound-related deaths occurred and toxicological effects were not observed.

A second study was then performed using B6C3F$_1$ mice, 10 per sex per group, to determine the doses to be used in a 2-year study; the animals were dosed with 250, 500, 1000, or 2000 mg/kg Acid Orange 3 (NTP 1988). The procedure followed was the same as above. Microscopic examination was performed on a number of tissues (see above) of all animals of the control and high-dose groups, of all animals that died prior to study termination, and on the kidneys of animals of the 500 and 1000 mg/kg dose groups. No compound-related deaths occurred. Final mean body weights of males and females of the 2000 mg/kg dose group were decreased by 12% and 11% of control values, respectively. Orange urine was observed for animals of the 1000 and 2000 mg/kg dose groups. Mild to severe nephropathy consisting of increased basophilia of the tubular epithelial cells, tubular dilatation, and cast formation were observed in 5 males and 2 females of the 1000 mg/kg dose group and in 10 males and 9 females of the 2000 mg/kg dose group.

Groups of 12 adult New Zealand White rabbits, 6 males and 6 females per group, were used to determine the percutaneous toxicity of a semipermanent hair dye formulation (P-24) containing 0.2% Acid Orange 3 (Burnett et al. 1976). One ml/kg of the mixture was applied undiluted twice weekly for 13 weeks to clipped sites on the dorsolateral aspect of the thoracic-lumbar area (one on each side of the midline), and the sites were alternated to minimize dermal irritation. The application sites on three animals per sex per group were abraded for the first dose of each week. The animals were restrained for 1 hour following dosing and the test site was then washed. Three groups of 12 negative-control animals were treated in the same manner as the test animals with the exception that no dye was applied.

All animals were weighed weekly. Hematological, clinical chemistry, and urinary determinations were made at study initiation and after 3, 7, and 13 weeks. All animals were killed after 13 weeks and examined grossly. Various organ-to-body weight ratios were determined and a number of tissues were examined

microscopically. No evidence of compound-induced toxicity was observed, no gross abnormalities were seen at necropsy, and no test article–related microscopic lesions were reported. No discoloration of the urine due to administration of the hair dye formulation was observed.

Chronic Oral Toxicity

Six male and six female purebred beagle dogs were fed for 24 months a composite material representative of a series of commercially available hair coloring products, which included the greatest concentration of each dye and each base component present in any of the formulations used; Acid Orange 3 was 0.24% of the formulation (Wernick, Lanman, and Fraux 1975). Two groups were fed 19.5 or 97.5 mg/kg/day of the test material; a control group was fed laboratory feed.

All animals were observed daily for toxicological and pharmacological effects. Body weights and feed consumption were determined weekly and daily, respectively. Physical examinations were conducted at study initiation and after 3, 6, 18, and 24 months. Hematological, clinical chemistry, and urinalysis parameters were determined on all animals of the control and high-dose groups and on three males and three females of the low-dose group at the same time. One male and one female animal of each group was selected for necropsy after 6, 12, and 18 months; all surviving animals were necropsied after 24 months. Selected organs were weighed, and organ-to-body weight ratios calculated. At the 24-month necropsy, liver and urinary bladder sections were taken from all animals for microscopic examination.

No significant toxicological or pharmacological effects were observed. No statistically significant differences were observed in body weight gain or in hematological or clinical chemistry values between the treated and control groups. All animals in both test groups excreted blue-brown colored urine daily; however, urinalysis did not detect any remarkable findings. No significant differences were observed in organ-to-body weight ratios between the treated and control groups, and no gross or microscopic lesions attributable to dosing were noted. All animals survived until study termination.

Photosensitization

Use of UV spectroscopy in the analysis of Acid Orange 3 (NTP 1988) suggests this ingredient absorbs in the UV region of the spectrum. Published data, however, on the photosensitization potential of Acid Orange 3 were not found.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Oral

Groups of 12 female New Zealand white rabbits were dosed by gavage on days 6 to 18 of gestation with the hair dye composite previously described in a chronic toxicity study using 0.24% Acid Orange 3 at a dose of 19.5 or 97.5 mg/kg/day, with the composite without the dyes at a dose of 97.5 mg/kg/day, or with 0.5% aqueous methylcellulose (vehicle) (Wernick, Lanman, and Fraux 1975). The dose volume for all groups was 1 ml/kg. All rabbits were killed on day 30 of gestation and various parameters were evaluated.

No teratogenic effects were observed in any of the groups. Fetal survival was not adversely affected by the dye-containing composite. Neither grossly abnormal fetuses nor soft tissue defects were observed. Animals of the high-dose group excreted blue-brown colored urine within an hour of dosing; urine color was normal the next day prior to dosing.

Groups of CFE-S rats, 20 males and 20 females per group, were mated, and gravid females were fed diet containing 1950 or 7800 ppm of the previously described dye composite that contained 0.24% Acid Orange 3 on days 6 to 15 of gestation; a control group was fed untreated feed throughout the study (Wernick, Lanman, and Fraux 1975). The female rats were weighed biweekly and killed on day 19 of pregnancy. Various reproductive and fetal parameters were examined.

No compound-associated adverse effects were observed for rats or the fetuses. No statistically significant dose-related effects were observed in the average number of implantation sites, live pups, early or late absorptions per litter, or number of females with one or more resorption sites. No gross abnormalities related to dosing were observed. The rats fed the test diet excreted blue-brown colored urine.

Groups of 10 male and 20 female Sprague-Dawley CD rats were fed the previously described dye composite that contained 0.24% Acid Orange 3 at concentrations of 1950 or 7800 ppm; a control group was fed untreated feed (Wernick, Lanman, and Fraux 1975). The study was divided into two parts. In Part I, the females received the basal diet for 8 weeks prior to mating and through weaning; the males were fed the test diet for 8 weeks prior to and during mating. In Part II, the females were fed the test diet 8 weeks prior to mating, during gestation and 21 days of lactation, whereas the males were fed untreated feed prior to and during mating. The remainder of the test procedure was the same for both parts of the study.

One gravid female of each group was killed for examination on day 13 of gestation. The remaining gravid dams were allowed to deliver; necropsy was performed on all dams that did not deliver to determine whether pregnancy had occurred. The pups were weighed at birth and after 4 and 21 days. At 21 days, all pups were killed and examined grossly.

No statistically significant dose-related differences in male or female fertility, length of gestation, number of females with resorption sites, live pups per litter, pup body weight, or pup survival were observed between the test and control groups in either part of the study. No significant differences in body weight gain or feed consumption were observed. No abnormal pups were noted. The rats dosed with the composite excreted blue-brown colored urine.

Dermal

Groups of 20 gravid Charles River CD rats were used to evaluate the teratogenic potential of a semipermanent hair dye formulation (P-24) containing 0.2% Acid Orange 3 (Burnett et al. 1976). The formulation was applied topically at a dose of 2 ml/kg to a shaved dorsoscapular area on days 1, 4, 7, 10, 13, 16, and 19 of gestation. (Pilot studies demonstrated that potential skin irritancy would not permit more frequent application.) Three negative control groups of rats were shaved but not dosed and rats of a positive control group were dosed orally with 250 mg/kg acetylsalicylic acid on days 6 to 16 of gestation. Feed and water were available ad libitum. All animals were weighed on the days of dosing and they were killed on day 20 of gestation.

The only reported observation was a change in color of the skin and hair at the site of application. No signs of toxicity were reported. Body weight gains and mean feed consumption were similar for animals of the treated and negative control groups. It was concluded that dermal administration of a semipermanent hair dye formulation containing 0.2% Acid Orange 3 "every third day of the gestation period produces no embryotoxic or teratogenic effects" in Charles River CD rats.

A multigeneration reproduction study was conducted using groups of 40 male and 40 female Sprague-Dawley rats that received topical applications of a semipermanent hair dye formulation (P-24) containing 0.2% Acid Orange 3 (Burnett et al. 1976, International Research and Development Corporation 1977). A dose of 0.5 ml was applied twice a week to a shaved area of the back that was approximately 1 inch in diameter. (The initial dose, 0.2 ml per application, was increased by 0.1 ml per application increments weekly until reaching 0.5 ml per application.) Successive applications were made to adjacent areas to minimize dermal irritation. Three negative control groups of rats were shaved but not dosed. When the rats were 100 days old, they were mated to produce an F_{1a} generation that was eventually used in a carcinogenicity study (summarized later in this report).

The F_0 generation was then reduced to 20 animals per group, remated to produce an F_{1b} generation, and then killed following weaning of the F_{1b} litters. Twenty male and 20 female rats per group were chosen from the F_{1b} litters and mated after 100 days to produce F_{2a} and F_{2b} litters. Five male and five female F_{1b} parents were necropsied after weaning of the F_{2b} litters.

Again following the same procedures, 20 male and 20 female F_2 parents per group were selected from the F_{2b} litters and mated to produce F_{3a}, F_{3b}, and F_{3c} litters. After weaning the F_{3b} litters, one weanling per litter per group was necropsied; the pups of the F_{3a} and F_{3c} litters were killed after weaning.

Parental generations were observed daily for changes in general behavior and appearance, and detailed observations were recorded weekly. Body weights and feed consumption were measured weekly. The pups were counted and weighed as a litter on days 0, 4, and 14 of lactation. On day 21 of lactation, the pups were counted, sexed, and examined for pharmacological effects.

Dermal reactions consisting of mild scabbing, fissuring, atonia, and a leathery texture occurred intermittently throughout the treatment period in each generation. No dose-related pharmacotoxicological signs were observed, and body weight gains, feed consumption, and survival were comparable for treated and control rats in each generation. During week 61, sialoadenitis was observed for some test and control animals; this regressed at week 63 but was followed by increased incidence of respiratory congestion in both test and control animals. The respiratory congestion persisted in the F_2 parents during the production of successive litters.

Litter size and pup body weights were similar for test and control groups. Fertility, gestation, survival, and live birth indices were comparable between test and control animals for the F_0, F_1, and F_2 parents. The F_2 parents had markedly reduced fertility indices for the three separate matings, but no significant differences were found between the control and test group with respect to fertility. The researchers did not report that the respiratory congestion was a significant factor in the reduction of fertility indices. The results of a special study established that the decreased fertility was due to reproductive tract changes in both the treated and control rats. No gross or microscopic treatment-related lesions were observed in F_{1b} parental rats or F_{3b} weanling rats. The topical application of a semipermanent hair dye formulation containing 0.2% Acid Orange 3 did not affect the reproductive performance of rats.

GENOTOXICITY

The mutagenic potential of Acid Orange 3 was evaluated using *Salmonella typhimurium* in a preincubation test (Zeiger et al. 1988). Concentrations of 10 to 2000 μg/plate Acid Orange 3 in DMSO were tested using *S. typhimurium* strains TA100, TA1535, TA97, and TA98 with and without metabolic activation. Vehicle was used as the negative control, sodium azide (TA100 and TA1535), 9-aminoacridine (TA97), and 4-nitro-*o*-phenylenediamine (TA98) were used as positive controls without metabolic activation, and 2-aminoanthracene was used as a positive control with metabolic activation. Acid Orange 3 was mutagenic.

The mutagenic potential of Acid Orange 3 was also evaluated in a standard transformation assay (Matthews 1986) without metabolic activation using A-31-1-13 BALB/c-3T3 cells (Matthews, Spalding, and Tennant 1993). Acid Orange 3, listed as a cytotoxic, mutagenic carcinogen, was tested at concentrations of 0.0278 to 0.222 mM and 0.0445 to 0.178 mM in two independent trials. Acid Orange 3 was active in the transformation assay.

CARCINOGENICITY

Oral

F344/N rats, 50 per sex per group, were used to determine the carcinogenic potential of Acid Orange 3 (NTP 1988). The

groups were given 375 or 750 mg/kg Acid Orange 3 in corn oil by gavage 5 days per week for 103 weeks. (The doses were determined based on the results of the previous subchronic toxicity study.) A control group was given vehicle. All animals were observed twice daily and the findings were recorded at least once monthly. Body weights were measured at study initiation, weekly for 13 weeks, and monthly thereafter. Microscopic examination was performed on a number of tissues from all animals (adrenal glands, aorta, brain, cecum, colon, costochondral junction, duodenum, esophagus, eyes, femur including marrow, heart, ileum, jejunum, kidneys, larynx including oral cavity, liver, lungs and bronchi, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity and turbinates, pancreas, parathyroids, pituitary gland, preputial or clitoral gland [after 1 June 1982] prostate/testes/seminal vesicles/epididymis/tunica vaginalis/scrotal sac or ovaries/uterus, rectum, salivary glands, sciatic nerve, skin, spinal cord, spleen, stomach, thigh muscle, thymus, thyroid gland, tissue masses, trachea, urinary bladder and Zymbal gland).

Mean body weights of males of the high-dose group were decreased 5% to 10% and 11% to 16% after 25 and 52 weeks, respectively, compared to the control group values. Mean body weights of females of the high-dose group were decreased 5% to 10% and 11% to 19% after weeks 47 and 70, respectively, compared to control group values. The survival of the males (after week 33) and the females (after week 14) of the high-dose group was significantly decreased compared to controls. By week 97, all males of the high-dose group died (6 deaths were accidental); 15 males of the low-dose group died nonaccidentally prior to study termination compared to 10 controls. Sixteen and 42 females of the low- and high-dose groups, respectively, died nonaccidentally compared to 7 controls.

The incidence of neoplasms was not increased for male rats of the high-dose group. Six transitional cell carcinomas originating from the transitional epithelium of the renal pelvis were observed in female rats of the high-dose group; this was a statistically significant increase compared to controls. A number of non-neoplastic renal lesions, including increased incidence and/or severity of nephropathy, hyperplasia of the pelvic epithelium, papillary necrosis, inflammation, and pigmentation, were observed for male and female animals. Mineralization, erosion of the epithelium, and ulcers in the glandular stomach and mineralization of the aorta occurred in some dosed rats; these lesions were attributed to renal failure. Parathyroid hyperplasia was increased in male rats of the high-dose group. Fibrous dysplasia of bones was thought to be secondary to renal disease and parathyroid hyperplasia. It was not clear whether chronic and suppurative inflammation of the colon and cecum observed in dosed male and female animals was a result of dosing or related to uremia from kidney failure. Incidences of interstitial cell hyperplasia were increased for dosed male rats as compared to controls, but the incidences of interstitial cell neoplasms were significantly decreased compared to controls.

The investigators concluded that under the conditions of this study, "there was no evidence of carcinogenic activity of C.I. Acid Orange 3 [Acid Orange 3] for male F344/N administered 375 mg/kg; because of a marked reduction in survival and no indication of carcinogenicity, the 750 mg/kg group was considered inadequate for assessment of carcinogenic activity. There was clear evidence of carcinogenic activity of C.I. Acid Orange 3 for female F344/N rats as shown by the occurrence of transitional cell carcinomas of the kidney in the 750 mg/kg group; this group had reduced survival and chemically related nonneoplastic lesions of the kidney."

A study using $B6C3F_1$ mice, 50 per sex per group, was performed according to the same procedures as above (NTP 1988), except that the gallbladder was added to the list of tissues examined. Males were given 125 or 250 mg/kg and females were given 250 or 500 mg/kg Acid Orange 3. Body weights were measured at study initiation, weekly for 12 weeks, and monthly thereafter.

Compared to control values, mean body weights of males of the high-dose group were decreased 6% to 10% from week 74 until study termination and mean body weights of males of the low-dose group were decreased 5% to 8% from weeks 44 to 70, after which the decrease was 9% to 14%. Mean body weights for females of the high-dose group were decreased 5% to 11% from week 74 until study termination and mean body weights for females of the low-dose group were decreased 5% to 8% from weeks 30 to 48, after which the decrease was 9% to 17%. Survival of test animals was similar to that of control animals. Twenty-three and 24 males of the low- and high-dose groups, respectively, died nonaccidentally, as compared to 15 controls and 27 and 26 females of the low- and high-dose groups, respectively, died nonaccidentally as compared to 27 controls.

A number of dose-related non-neoplastic renal lesions, including increased incidence and/or severity of inflammation, fibrosis, nephrosis, papillary degeneration, medullary (papillary) necrosis, tubular dilatation, tubular mineralization, and lymphoid hyperplasia, were observed. Epithelial hyperplasia was observed in no control, one low-dose and three high-dose females, and a squamous cell carcinoma was observed in one low-dose female. Hemangiosarcomas were observed in six control, one low-dose and five high-dose males. Squamous cell papillomas of the nonglandular stomach were observed in four control but not low- or high-dose females.

The investigators concluded "there was no evidence of carcinogenic activity of C.I. Acid Orange 3 for male $B6C3F_1$ mice administered 125 or 250 mg/kg or for female $B6C3F_1$ mice administered 250 or 500 mg/kg. Nonneoplastic lesions of the kidney were observed in both dose groups of both sexes of rats and mice."

Dermal

F_{1a} generation Sprague-Dawley rats from the previously described reproduction study were used to determine the

carcinogenic potential of a semipermanent hair dye formulation (P-24) containing 0.2% Acid Orange 3 (Burnett et al. 1976; International Research and Development Corporation 1979). Twice a week, a dose of 0.5 ml of the hair dye formulation was applied topically to a shaved 1 inch diameter area of the back of 120 rats, 60 per sex, for 12 months. (The initial dose was 0.2 ml per application, which was increased by 0.1 ml per application increments weekly until reaching 0.5 ml per application.) Successive applications were made to adjacent areas to minimize dermal irritation. Three negative control groups of 120 rats were shaved but not dosed. The rats were observed daily for signs of toxicity and mortality; detailed observations were recorded weekly. Body weights were determined weekly for the first 14 weeks and monthly thereafter; feed consumption was determined weekly. Biochemical measures were determined from blood and urine samples that were collected from five male and five female fasted rats per group at 3, 12, 18, and 24 months. Five males and five females per group were killed after 12 months.

No signs of toxicity were observed. Test animals had a slightly greater incidence of skin lesions at various locations, including ulceration, scabbing, abscessation, and thickening, than did control animals. Coloration of the hair and skin at the application site was observed in several treated animals but was not considered to be pathologically significant. Body weight gains, survival, hematological values, and biochemical measures were similar for animals of the treated and control groups. After 3, 12, and 24 months, the animals consistently had dark straw-colored urine, with three and nine animals having a dark brown urine at 12 and 18 months, respectively.

The incidence of enlarged and/or firm livers was slightly greater in the test group as compared to the controls; this was considered "possibly compound related." Other lesions considered "possibly compound related" for males and females of the test group include a proportionately greater number of animals with parathyroid gland hyperplasia, greater frequency of hepatocellular hypertrophy or hyperplasia, and a considerably increased incidence of hyperkeratosis and dermatitis from a variety of locations. Several male test animals had hyperkeratosis and/or acanthosis involving the gastric mucosa, which was also "possibly compound related."

The incidence of hematopoiesis in the livers of test animals was somewhat greater than that of all controls; the significance of this increase was not determined. For female test animals, the incidence of pituitary adenomas was significantly increased as compared to females from two of the three control groups and the incidences of mammary adenocarcinoma/mammary carcinoma were significantly increased as compared to females in one of the three control groups; however, these differences were not considered biologically significant. Actuarial (life table) analyses did not indicate significant variations in indices of tumor bearing in the test animals as compared to the control groups by sex.

A 23-month skin painting study was performed using groups of 50 male and 50 female Eppley Swiss Webster mice to determine the carcinogenic potential of a semipermanent hair dye formulation (P-24) containing 0.2% Acid Orange 3 (Burnett et al. 1980). A 0.05-ml sample of the test solution was applied undiluted to a 1-cm^2 area of clipped skin of the interscapular region. A group of negative controls was shaved but not dosed. Observations were made daily and body weights were determined monthly. After 9 months, 10 male and 10 female animals from each group were necropsied, with liver and kidney weights being determined. Gross and microscopic examinations were made for all animals found dead, killed due to moribund condition, or killed at study termination.

After 9 months, relative and absolute liver and kidney weights were not significantly different from control values. No compound-induced neoplasms were observed. A semipermanent hair dye formulation containing 0.2% Acid Orange 3 applied dermally for 23 months did not have a carcinogenic effect.

CLINICAL ASSESSMENT OF SAFETY

Dermal Irritation/Sensitization

Published data on the clinical dermal irritation and/or sensitization potential of Acid Orange 3 were not found.

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 females aged 15 to 60.

A number of epidemiological 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 International Agency for Research on Cancer (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 to 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 epidemiological 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

Acid Orange 3 is a nitro color that functions as a hair colorant in four hair dyes and colors. The hair dyes containing Acid Orange 3, 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 of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The following caution statement should be displayed conspicuously on the labels of coal tar hair dyes:

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

In oral toxicity studies, adverse effects were not observed in rats dosed with ≤1500 mg/kg or mice dosed with ≤1000 mg/kg Acid Orange 3 for 14 days, lesions of the kidneys were observed in rats dosed with 1500 mg/kg and in mice dosed with 1000 or 2000 mg/kg for 13 weeks, and adverse effects were not observed in dogs fed ≤97.5 mg/kg/day of a hair coloring product containing 0.24% Acid Orange 3. In a dermal study, adverse effects were not observed for rabbits dosed twice weekly for 13 weeks with a hair dye formulation containing 0.2% Acid Orange 3.

Teratogenic or embryotoxic effects were not observed in a dermal study in which gravid rats received applications of a hair dye formulation containing 0.2% Acid Orange 3 every third day of gestation, and reproductive effects were not observed in a multigeneration study in which rats received topical applications of a hair dye formulation containing 0.2% Acid Orange 3. In oral studies, teratogenic or reproductive effects were not observed for rabbits or rats dosed with a hair dye formulation containing 0.24% Acid Orange 3.

Acid Orange 3, tested at concentrations of ≤2000 μg/plate, was mutagenic to *S. typhimurium* in a preincubation test and it was active in a transformation assay without metabolic activation using BALB/c-3T3 cells when tested at concentrations ≤0.222 mM.

In a dermal carcinogenicity study in which rats received twice weekly applications of a hair dye formulation containing 0.2% Acid Orange 3, "possibly compound related effects" included enlarged and/or firm livers and an increase in parathyroid gland hyperplasia, hepatocellular hypertrophy or hyperplasia, hyperkeratosis and dermatitis, and hyperkeratosis and/or acanthosis involving the gastric mucosa. A carcinogenic effect was not observed for mice used in a 23-month skin painting study of a hair dye formulation containing 0.2% Acid Orange 3. In oral carcinogenicity studies in which rats were dosed with ≤750 mg/kg and mice were dosed with ≤500 mg/kg Acid Orange 3, 5 days/week for 103 weeks, clear evidence of carcinogenic activity was observed for female rats as evidenced by transitional cell carcinomas of the kidney, but no evidence of carcinogenicity was observed for male rats, male mice, or female mice; non-neoplastic lesions of the kidney were observed for male and female rats and mice.

DISCUSSION

Acid Orange 3 has mutagenic potential, but a carcinogenic effect was not seen in studies in which rats and mice received dermal applications of a hair dye formulation containing 0.2% Acid Orange 3. Also, a hair dye formulation containing 0.2% Acid Orange 3 was not a reproductive toxin upon dermal or oral administration to rats and rabbits. Because it is not known at what concentrations cosmetic companies are using this ingredient, a maximum allowable concentration of 0.2% was determined from these test data.

The Expert Panel recognizes that irritation and sensitization data on Acid Orange 3 are absent from this report. However, the hair dyes containing Acid Orange 3, 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 of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Expert Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures.

CONCLUSION

On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes that Acid Orange 3 is safe for use in hair dye formulations at concentrations ≤0.2%.

REFERENCES

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

Burnett, C., E. I. Goldenthal, S. B. Harris, et al. 1976. Teratology and percutaneous toxicity studies on hair dyes. *J. Toxicol. Environ. Health* 1:1027–1040.

Burnett, C., M. M. Jacobs, A. Seppala, and P. Shubik. 1980. Evaluation of the toxicity and carcinogenicity of hair dyes. *J. Toxicol. Environ. Health.* 6:247–257.

Cosmetic, Toiletry, and Fragrance Association (CTFA). 1993. Personal correspondence from G. N. McEwen. (Dated 6 December 1993.)[2]

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

Elder, R. L., ed. 1985. Final report on the safety assessment of *p*-Phenylenediamine. *J. Am. Coll. Toxicol.* 4:203–266.

European Economic Community. 1995. *EEC Cosmetic Directive 76/768/EEC, as amended, Annexes I through VII.* Brussels: EEC.

Food and Drug Administration (FDA). 1984. Cosmetic product formulation and frequency of use data. FDA computer printout. Washington, DC: FDA.

FDA. 1992. Modification in voluntary filing of cosmetic product ingredient and cosmetic raw materials composition statements. *Fed. Register* 57:3128–3130.

[2]Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

FDA. 1997. Frequency of use of cosmetic ingredient. FDA computer printout. Washington, DC: FDA.

International Agency for Research on Cancer (IARC). 1993. *IARC monographs on the evaluation of carcinogenic risks to humans*, Vol. 57, 121–127, 159–165. Lyon, France: IARC.

International Research and Development Corporation. 1977. Multigeneration reproduction study in rats on formulations P-21, P-22, P-23, and P-24. Study No. 355-002b. Report dated November 4. Unpublished data submitted by Clairol. (148 pages.)[2]

International Research and Development Corporation. 1979. Lifetime toxicity/carcinogenesis study in rats on formulations P-21, P-22, P-23, and P-24. Study No. 355-003b. Report dated April 10. Unpublished data submitted by Clairol. (554 pages.)[2]

Matthews, E. J. 1986. Assessment of chemical carcinogen-induced transforming activity using BALB/c-3T3 cells. *J. Tissue Culture Methods* 10:157–164.

Matthews, E. J., J. W. Spalding, and R. W. Tennant. 1993. Transformation of BALB/c-3T3 cells: V. Transformation responses of 168 chemicals compared with mutagenicity in *Salmonella* and carcinogenicity in rodent bioassays. *Environ. Health Perspect.* 101(Suppl 2):347–482.

National Toxicology Program (NTP). 1988. *NTP technical report on the toxicology and carcinogenesis studies of C.I. Acid Orange 3 (CAS No. 6373-74-6) in F344/N rats and B6C3F$_1$ mice. (Gavage studies.)* NTP TR 335. NIH Publication No. 89-2591. Research Triangle Park: NTP.

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

Rempe, J. M., and L. G. Santucci. 1997. *CTFA list of Japanese cosmetic ingredients*, 3rd ed. Washington, DC: CTFA.

Society of Dyers and Colourists. 1971. *Colour index*, 3rd ed., Vol. 4. Bradford, UK: Society of Dyers and Colourists, with acknowledgment to the American Association of Textile Chemists and Colourists, 4007.

Ventura, F., A. Figueras, J. Caixach, D. Fraisse, and J. Rivera. 1989. FAB mass spectrometry of sulphonated dyes. Application to water analysis. *Fresenius Z. Anal. Chem.* 335:272–278.

Wenninger, J. A., R. C. Canterbery, and G. N. McEwen, Jr., eds. 2000. *CTFA international cosmetic ingredient dictionary and handbook*, 8th ed., Vol 1., 16–17. Washington, DC: CTFA.

Wernick, T., B. M. Lanman, and J. L. Fraux. 1975. Chronic toxicity, teratologic, and reproduction studies with hair dyes. *Toxicol. Appl. Pharmacol.* 32:450–460.

Zeiger, E., B. Anderson, S. Haworth, T. Lawlor, and K. Mortelmans. 1988. *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. Mol. Mutagen.* 11(Suppl 12):1–158.

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Final Report on the Safety Assessment of *N,N-Bis*(Hydroxyethyl)-*p*-Phenylenediamine Sulfate

N,N-Bis(Hydroxyethyl-_p_-Phenylenediamine Sulfate is used in cosmetic hair dye and color formulations at concentrations ≤5%.

The oral LD_{50} of _N,N-Bis_(Hydroxyethyl-_p_-Phenylenediamine Sulfate for rats was 264 mg/kg. Rats fed NNPD at ≤0.30% for 13 weeks had decreased body weights, feed consumption, serum iron concentrations, and reduced pituitary weights. The results from subchronic dermal studies at 1.0% were unremarkable.

In sensitization studies using guinea pigs, 3% NNPD induced delayed hypersensitivity. A 2% solution produced a strong contact allergic, but not a photoallergenic, response. A repeated insulted patch test in 104 humans produced definite sensitization in one subject and possible sensitization in another.

Neither teratogenic nor embryotoxic effects were produced in rats by dermal administration of 1.0% NNPD or by administration of 0.3% NNPD in the diet. When assayed in rats, NNPD was neither hepatocarcinogenic nor a promoter of liver cancer.

On the basis of the data presented in this report, and the placement of precautionary labeling on cosmetic hair dye products containing coal tar ingredients, it is concluded that _N,N-Bis_(Hydroxyethyl)-_p_-Phenylenediamine Sulfate is safe as a cosmetic ingredient in the present practices of use and concentration.

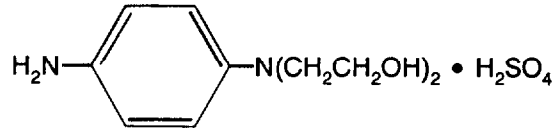
INTRODUCTION

N,N-Bis(2-Hydroxyethyl)-_p_-Phenylenediamine Sulfate is a substituted aromatic amine that is used in hair dyes and colors.[1] It is a derivative of _p_-Phenylenediamine[2] and is an oxidative hair dye. The final report on the safety assessment of _p_-Phenylenediamine can be found in the *Journal of the American College of Toxicology*, Vol. 4, No. 3.[3]

CHEMISTRY

Definition and Structure

N,N-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate (CAS No. 54381-16-7; 57524-61-5; 58262-44-5) is the substituted aromatic amine that conforms to the formula:[4]



N,N-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate is also known as 2,2'-[(4-Aminophenyl)Imino]Bisethanol Sulfate (Salt) and Ethanol 2,2'-[(4-Aminophenyl)Imino]Bis-, Sulfate (Salt).[4]

PROPERTIES

N,N-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate is light gray or pink and is in the form of a powder or crystals.[5] It has a melting point of 163–171°C. Properties of *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate are summarized in Table 1.

METHOD OF MANUFACTURE

The method of manufacturing *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate is proprietary.[6]

Analytical Methods

High-performance liquid chromatography (HPLC) has been used to assay *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate.[5]

TABLE 1. PHYSICAL AND CHEMICAL PROPERTIES

		References
Color	Light gray or pink	5
Appearance	Powder or crystals	5
Molecular formula	$C_{10}H_{16}N_2O_2 \bullet H_2O_4S$	4
	$C_{10}H_{18}O_6N_2S$	5
Molecular weight	294.33	5
Melting point	163–171°C	5
% Assay	95% minimum	5
% Coupling assay	95% minimum	5
Impurities		
Iron	100 ppm, maximum	5
Ash	2%, maximum	5
Inorganic salts, water		7

Impurities

N,N-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate was found to have a minimum purity of 95%.[5] It contained inorganic salts as manifested by 2% ash, a maximum of 100 ppm iron, and water.[7] Data regarding the presence of organic chemicals as impurities were not available.

USE

Cosmetic

The product formulation data that were submitted to the Food and Drug Administration (FDA) in 1984 stated that *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate was used in a total of 183 hair dye and color preparations at a concentration of ≤5% (Table 2).

The FDA cosmetic product formulation computer printout[8] is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations.[9] Ingredients are listed in preset concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration would be a fraction of that reported to the FDA. Data submitted within the framework of present concentration ranges provide the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to ten-fold error in the assumed ingredient concentration.

The oxidative or permanent hair dyes containing *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate, as oxidative hair dye products,[3] 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.[10] In order to be exempt, the following caution statement must be displayed on all oxidative hair dye products:

> Caution—this product contains ingredients which may cause skin irritation on certain individuals and a preliminary test according to accompanying direc-

TABLE 2. PRODUCT FORMULATION DATA[8]

Product category	*Total no. containing ingredient*	*No. of product formulations within each concentration range (%)*		
		1–5	*0.1–1*	*≤0.1*
Hair dyes/colors (requiring caution statements)	183	9	55	119
1984 Totals	183	9	55	119

tions should be made. This product must not be used for dying the eyelashes or eyebrows; to do so may cause blindness.

Patch test instructions call for a 24-h patch on the skin of the user with the intermediates and hydrogen peroxide mixed in the same manner as in use. This test is to be performed prior to each and every application of the hair dye.[11]

ANIMAL TOXICOLOGY

Acute Toxicity

Oral

N,N-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate, given orally as a 0.01% solution in 10% dimethylsulfoxide (DMSO) to male rats, strain and number unspecified, had an LD_{50} of 264 mg/kg.[7]

Short-Term Toxicity

Oral

Four groups of Sprague-Dawley rats, 8 males and 8 females per group, were fed a diet containing 0.01–0.5, 0.03, 0.1, or 0.3% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate for 5 weeks.[12] The first group was fed a diet containing 0.01% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate for 2 weeks; the diet was then changed to contain 0.5% of the ingredient for the remainder of the study. A control group of 8 males and 8 females were fed untreated feed for 5 weeks. Two additional groups, 10 males per group, were fed a diet containing either 0.5 or 0.75% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate for 2 weeks. The control group of 9 male rats was fed untreated feed for 2 weeks.

All animals were weighed at study initiation; body weights and feed consumption were measured weekly. Animals were checked daily for mortality; clinical observations were made weekly. Upon study completion, all animals were fasted 24 h prior to being killed and necropsied.

Body weights of male rats fed 0.75% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate for 2 weeks were significantly reduced during the study when compared to controls; no other significant differences in body weight were observed during the study for any other group. At the time of study termination, body weights of the rats fed 0.5 and 0.75% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate for 2 weeks were significantly reduced when compared with the controls. Feed consumption was reduced for these two groups during both weeks of dosing. The male rats of the 0.75% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate group appeared highly hyperactive; no clinical signs were observed in any other group.

At necropsy, the kidneys of many rats appeared either pale or dark, and the livers of three male rats fed 0.3% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate appeared enlarged. When compared with control values, liver to body weight was significantly increased in the 0.01–0.5% group females and the 0.3% group males and females (dosed for 5 weeks) and absolute liver weight was significantly decreased in the 0.75% group (males dosed for 2 weeks). Kidney to body weight was significantly increased in female rats fed 0.01–0.5, 0.03, 0.01, and 0.3% *N,N-Bis*(2-Hydroxyethyl)-

p-Phenylenediamine Sulfate and a concomitant decrease was observed in kidney weight and kidney to body weight in male rats fed 0.75% of the test compound. The decrease in organ weight observed in the male rats fed 0.5 and 0.75% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate was attributed to diet rejection at these concentrations.

Subchronic Toxicity

Oral

Three groups of Sprague-Dawley rats, 40 males and 45 females per group, were fed diet containing 0.03, 0.10, or 0.30% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate for 13 weeks.[7] (Dose concentrations were determined based on the results of a previous short-term diet study.)[12] A control group, 40 males and 45 females, received untreated feed.

Animals were checked daily for mortality; clinical observations were made weekly. Body weights and feed consumption were determined weekly.

After 6 weeks of dosing, 5 males and 5 females were randomly selected from the control and high-dose groups for determination of methemoglobin concentration. Five similar nontest rats were given at intraperitoneal (i.p.) injection of 400 mg/kg phenacetin and used as positive controls for the methemoglobin testing.

After a minimum of 13 weeks, 10 males and 10 females randomly selected from each group were fasted for 24 h and killed; clinical chemistry values and hematological parameters were measured and gross and microscopic examinations were performed. All remaining rats were fed their respective diets and used for further study.

Neither mortality nor signs of toxicity were observed during the 13 weeks of dosing. When compared with the controls, males of the high-dose group had significantly reduced body weights every week except weeks 1, 10, and 11 and females of the high-dose group had significantly reduced body weights at weeks 11 and 12. Significant decreases in feed consumption were observed for males of the high-dose group at weeks 3, 7, and 12 when compared with controls; no significant differences were observed in feed consumption by females of the high-dose group.

Methemoglobin values were below the limit of detection for all animals tested after 6 weeks except a control male with a value of 0.8 g/100 ml, two control females with values of 0.1 g/100 ml and 0.2 g/100 ml, and a high-dose male with a value of 0.1 g/100 ml; these values were much lower than the values obtained for the positive controls.

The only dose-related abnormality observed at necropsy was darkened thyroid glands in 6/9 males and 7/11 females of the high-dose group and in 1/10 males of the mid-dose group. High-dose males had significantly reduced pituitary gland weights compared with the controls.

There was no significant difference in any of the hematological parameters of any of the test rats compared with control values. Male rats of the 0.10 and 0.30% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate groups had significantly decreased serum iron concentrations compared with the controls; no other clinical chemistry values differed significantly. No microscopic changes due to *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate administration were observed.

Dermal

An oxidative hair dye formulation containing 1.0% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate and an autoxidative hair dye formulation containing 0.5%

N,N-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate were applied topically, twice weekly for 13 weeks to two groups of 12 New Zealand White rabbits, 6 males and 6 females per group.[13] The oxidative hair dye formulation was mixed with an equal volume of 6% hydrogen peroxide prior to application and the autoxidative formulation was used as provided.

A dose of 1 ml/kg was applied to shaved areas on the dorsolateral aspects of the thoracic-lumbar area, on each side of the midline; application sites were altered to minimize irritation. Test sites of three animals/gender/group were abraded on the first treatment day of each week. Three negative control groups of 12 rabbits/group were treated in the same manner as the test groups, with the exception that no dye was applied.

All rabbits were weighed weekly; clinical chemistry, including hematological and renal function parameters, was examined at the beginning of the study and at 3, 7, and 13 weeks. At the end of 13 weeks, all animals were necropsied. Organ-body weight ratios were determined and a microscopic examination of some tissues was performed.

No clinical signs of toxicity due to test substance administration, such as urine discoloration or mortality, were observed in either group. Body weight gains of the test groups were at least equal to those of the controls. In some animals, particularly those of the group treated with the oxidative hair dye formulation, a slight thickening of the treated skin was observed; this was not unexpected due to dose frequency.

Relative organ to body weights may have been statistically different than the combined value of the three control groups, but no significant difference was observed when test group weights were compared with individual control group values; the differences were not accompanied by histomorphological evidence of toxicity.

Statistical differences between test and control groups were observed for some clinical chemistry and hematological values; these differences were not considered toxicologically significant. Burnett et al.[13] did not observe gross nor microscopic lesions due to test substance administration.

Chronic Toxicity

Oral

Male Sprague-Dawley rats, 10 per group, that were used in a subchronic feeding study previously summarized[7] were fed their respective diets containing 0, 0.03, 0.10, or 0.30% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate for an additional 3 months following the initial 13 week dosing period.[7] Twenty male Sprague-Dawley rats per group from the same previous study (which were also used in a dominant lethal study which will be summarized later) were fed their respective diets containing *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate for 20 weeks and then control feed for 6–8 weeks.

Animals were checked daily for mortality; clinical observations were made weekly. Body weights and, except for the recovery animals, feed consumption were determined weekly. After a minimum of 26 weeks on study, all rats were fasted for 24 h and killed. Clinical chemistry values and hematological parameters were measured for the continuously fed rats and for 10 randomly selected recovery rats.

Test compound-related changes were not observed during the second 13 weeks of dosing. Animals of the continuously exposed high-dose group weighed consistently, but insignificantly, less than the controls. Feed consumption by the animals of the continuously exposed high-dose group was also consistently less than that of the

controls, but the difference was significant only at week 25. During dose week 15–18, the males of the recovery high-dose group weighed significantly less than the controls; the random sampling may have been responsible for this difference in results. After being fed control diet, the mean body weights of the high-dose recovery males did not differ significantly from the controls, although they remained consistently lower.

At necropsy, 4 of 20 mid-dose recovery males had slightly dark thyroid glands, 1 of 20 and 17 of 20 high-dose recovery males had slightly dark and dark thyroid glands, respectively, and 10 of 10 continuously exposed high-dose males had dark thyroid glands. There were no other gross effects observed related to treatment. Kidney weights of continuously exposed males in all groups and the relative heart to body weights of continuously exposed high-dose males were significantly increased compared with the controls; these differences were attributed to decreased body weights when compared with the controls and were considered not of toxicological importance. No significant difference in organ weight was observed in animals of the recovery group when compared with the controls.

Hematocrit concentrations were significantly decreased in the continuously exposed low-dose group and serum phosphate concentrations were significantly increased in the low-dose recovery male groups when compared with control values. A microscopic examination was not performed.

Female Sprague-Dawley rats, 10 per group, that were used in a subchronic feeding study previously summarized[7] were fed their respective diets containing 0, 0.03, 0.10, or 0.30% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate feed for an additional 3 months following the initial 13 week dosing period.[7]

Mortality was assessed daily. Clinical observations, body weights, and feed consumption were determined weekly. After a minimum of 26 weeks of dosing, all rats were fasted for 24 h and killed. Clinical chemistry values and hematological parameters were measured.

No clinical signs of toxicity were observed. One high-dose female died; the death was not considered compound-related. High-dose females had consistently, but insignificantly, lower body weight and feed consumption values than the controls.

At necropsy, 2 of 10 and 1 of 10 females of the mid-dose group had slightly dark and dark thyroid glands, respectively, and 1 of 9 and 8 of 9 high-dose-females had slightly dark and dark thyroid glands, respectively. No significant differences were observed in organ weight between treated and control animals.

No significant difference between groups was observed for any clinical chemistry or hematology values. A microscopic examination was not performed.

Sensitization

Fifteen female Hartley albino guinea pigs were used to determine the sensitization potential of *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate using the Schultz Method Modified.[14] An approximately 36 cm^2 area of the left flank of each animal was shaved and 0.5 ml of a 3% solution of *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate in Schultz Vehicle II was applied to a 6.5 cm^2 area. Each animal was dosed for 5 consecutive days/week for 3 weeks, observed after the initial induction dose, and scored according to the Draize method. Each animal served as its own control to assure that the challenge reaction was not due to skin irritation.

The number of reactors to 3% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate was 5 of 15 and the primary irritation index (PII) was 0.38. A solution of 3%

N,N-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate induced delayed hypersensitivity in guinea pigs.

Fifteen female Hartley albino guinea pigs were used to determine the sensitization potential of *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate using the Schultz Method Modified.[15] An approximately 36 cm^2 area of the left flank of each animal was shaved and 0.5 ml of a 3% solution of *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate in Schultz Vehicle II was applied to a 6.5 cm^2 area. Each animal was dosed 5 consecutive days/week for 3 weeks.

At the beginning of week 4, an additional three doses of test compound were applied on three consecutive days. All animals were observed daily and observations and body weight changes were noted weekly during dosing.

Two weeks after the last dose, an area on the right flank of each animal was shaved and a challenge dose of 0.5 ml of test substance was applied. The challenge site was scored, following the Draize method, for erythema and edema at 24, 48, and 72 h after dosing. Five untreated female guinea pigs were dosed with a single application of 3% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate at the time of challenge and served as controls.

Two weeks after the initial challenge, all test and control animals were challenged a second time on the upper left flank. Twelve days after the second challenge, each animal was challenged on the dorsal midline with 3% *p*-Phenylenediamine and observed for 72 h following dosing. The animals were challenged a third time on the upper right flank with *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate 48 h after being challenged with *p*-Phenylenediamine.

After several test compound applications, all test animals developed slight skin irritation; a reddish-brown discoloration of the skin was observed. Well-defined erythema was observed for one animal after 1 week; this was followed by eschar and thickening of the skin. One animal died on study; death was not attributed to treatment.

The number of positive reactions after challenges 1, 2, and 3 with *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate was 6, 9, and 7, respectively. Several control animals developed slight redness after test substance application; therefore, challenged test animals with slight redness were considered to have skin irritation reactions. The results of the challenge with *p*-Phenylenediamine were negative; one animal developed well-defined redness, but this animal appeared to be highly reactive to all dosing. *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate, 3%, induced delayed hypersensitivity in guinea pigs while a single dose of *p*-Phenylenediamine, 3%, did not induce a gross sensitivity reaction.

Photosensitization

Hartley albino guinea pigs were used to determine the photosensitization potential of *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate.[16] A dose of 2% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate (higher concentrations had poor solubility) in 80% DAE 433 and 20% physiological saline (vehicle) was used for induction; the test group consisted of 8 male and 8 female guinea pigs. A positive control of 5% musk ambrette in vehicle was used. The nuchal areas of all test and positive control animals were shaved and depilitated 24 h prior to induction; all animals were shaved daily during induction and challenge.

The light source was a 150 W Xenon Lamp which emitted UVA, UVB, and visible light waves; during induction, a WG-354 glass filter was used to remove UVB waves.

The minimum erythemal dose (MED) for UVA and UVB, which is the time necessary for a given light source to cause a barely perceptible reddening after irradiation, was determined by shaving and depilating the left flank of four guinea pigs 2 h prior to irradiation; the shaved area was irradiated with UVA for 14 and UVB for 60, 90, and 120 sec. The MED was 14 min and 90 sec for UVA and UVB, respectively.

During week 1 of induction, 0.1 ml of test substance was applied to a 1.8 cm diameter test site on the nuchal area; applications were made for four consecutive days. One hour after dosing, the test sites were irradiated with 50% MED of UVA. Skin irritation was scored daily, according to the Draize method, 24 h after dosing.

During weeks 2 and 3 of induction, all animals were dosed on the same sites with 0.1 ml of test material for four consecutive days. One h after dosing, all test sites were irradiated with 1 MED of UVB. On the first and third day of weeks 2 and 3, all animals were given intradermal injections of 0.1 ml Freund's Complete Adjuvant in physiological saline (1:1) on four different areas surrounding the test site.

Two weeks after the last week of induction, a challenge was performed to determine photocontact and contact sensitization. Each test animal was challenged with 0.1 ml of 1% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate over a 1.8 cm diameter site of the left lumbar area for three consecutive days; the controls were challenged with 5% musk ambrette. All test sites were irradiated with 50% MED of UVB 1 h after dosing and each site was scored for irritation 24 h after dosing. On the left lumbar area below the previous site, the same procedure was carried out for three consecutive days, with the exception that the test sites were irradiated with 50% MED of UVA 1 h after dosing. The test sites were scored for irritation 24 h after dosing.

In order to determine the extent of contact sensitization, the same procedure was carried out at a site adjacent to the first site, but the second site was not exposed to UV light. Again the sites were scored for irritation 24 h after dosing.

Neither erythema nor edema due to dosing was observed during induction; significant irritation was observed in the area of injection of Freund's Complete Adjuvant. During the 72 h observation period following the challenge; 16 of 16 guinea pigs responded (had an irritation score of ≥ 1) to all three doses, all animals had well-defined irritation at all test sites upon challenge with 1% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate. *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate did not produce a photoallergic response; however, it did produce a strong contact allergic response.

Reproductive/Teratogenic Effects

Twenty-five female Sprague-Dawley rats/group that were used in a previous subchronic oral study[7] were used in a teratology study to determine the teratogenic effects of *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate.[7] All female rats were fed control feed during mating with untreated males. As of day 0 of gestation, the female rats were again fed their appropriate test diet containing 0, 0.03, 0.1, or 0.3% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate.

Each animal was observed daily for mortality and signs of toxicity. Body weights were measured on days 0, 6, 9, 12, 15, and 20 of gestation. Feed consumption of 10 dams/group was measured on days 11 and 19 of gestation. On day 20 of gestation, all rats were killed and litter and reproduction parameters were evaluated. The abdominal and thoracic cavities of each female were examined.

The high-dose females consistently, but insignificantly, had lower body weights

and body weight gains than did the controls throughout gestation. On day 19, feed consumption by the low-dose females was significantly greater and by the high-dose females was significantly less than the controls.

The only significant difference observed was a reduced body weight of fetuses of the low-dose group as compared with the controls; this difference was small and probably a chance effect. There was no dose-response effect observed in the higher dose groups. No differences in skeletal and visceral abnormalities were found between fetuses of the control and test groups.

There were no significant differences in gravid uteri weights. The pregnancy rates were 92, 96, 80, and 76% for the control, low-, mid-, and high-dose groups, respectively. Oral administration of ≤0.3% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate produced neither embryonic nor teratogenic effects.

Charles River CD rats, 20 gravid rats/group, were used to determine the teratogenic potential of an autoxidative hair dye formulation containing 0.5% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate and an oxidative hair dye formulation containing 1.0% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate.[13] One positive and three negative control groups were used.

The dorso-scapular area of each test rat was shaved the day before dosing; the autoxidative formulation was applied and the oxidative formulation was mixed with an equal volume of 6% hydrogen peroxide prior to application. The test rats were given dermal applications of 2 ml/kg of test substance on days 1, 4, 7, 10, 13, 16, and 19 of gestation. The negative controls were shaved but not dosed; the positive controls were dosed orally by gavage with 250 mg/kg acetylsalicylic acid on days 6–16 of gestation.

Neither signs of toxicity nor irritation were observed due to test substance application; changes in skin and hair color were seen at the application site. No significant differences in body weight or feed consumption were observed between the treated and negative control groups. Positive control rats had a marked reduction in weight gain throughout gestation and a moderate decrease in feed consumption during days 7–13 of gestation when compared with the treated and control rats.

All rats were killed on day 20 of gestation and reproduction and litter parameters were evaluated; no significant differences were observed between the test groups and the negative controls. Dermal application of dye formulations containing 0.5 or 1% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate produced neither embryotoxic nor teratogenic effects.

Twenty male Sprague-Dawley rats/group that were used in a previous subchronic oral study[7] were used in a dominant lethal study for mating and examination of fetal loss due to *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate.[7] Male rats were fed their appropriate diet containing 0, 0.03, 0.1, or 0.3% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate for a total of 20 weeks. All groups were then fed control diet and mated with untreated female Sprague-Dawley rats.

Each male rat was placed with two females. Rats were observed daily for evidence of mating; if mating did not occur, the males remained with the same females for a total of 7 days. After 7 days, the mating procedure was repeated with two new untreated females per male.

Each male was observed daily for mortality and weekly for signs of toxicity. Females were observed daily and body weights were measured on days 0, 12, and 17 of gestation. Gravid females were killed on day 17 ± 1 of gestation and litter and reproduction parameters were evaluated. Females that did not have evidence of mating were killed on day 17 ± 1 from the midpoint of mating.

As noted in the chronic study,[7] the mean body weights of the high-dose males were consistently less than the mean body weights of the controls; this difference was only significant during weeks 15–18. Each male was successfully mated with at least one female during the 2 weeks of mating. There was no significant difference in body weight between the female groups during mating. No significant differences were observed in any of the reproduction or litter parameters evaluated. Oral administration of ≤0.3% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate did not produce a dominant lethal effect.

CARCINOGENICITY

A total of 250 male F344/DuCrj rats were used in an *in vivo* medium-term bioassay system[17–19] to examine the potential promoting effects of *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate on hepatic carcinogenesis as well as its carcinogenic potential.[2] The rats were divided into three groups, with groups 1 and 3 being divided into four subgroups (number of rats/group or subgroup not specified).

Groups 1 and 2 were given an i.p. injection of 200 mg/kg *N*-nitrosodiethylamine (DEN), a γ-GT-positive foci initiator. Two weeks after DEN injection, the subgroups in group 1 were fed either 110, 330, or 1000 ppm *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate or 600 ppm of the positive control, 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB).

Group 2 served as a control group in determining the number of γ-GT-positive foci due to *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate as compared with those due to DEN alone.

Group 3 was given an i.p. injection of saline, instead of DEN, 2 weeks prior to being dosed; the group 3 subgroups were dosed in the same manner as the group 1 subgroups. This group was used to estimate the carcinogenic potential of *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate.

A two-thirds partial hepatectomy was performed on all animals 3 weeks after study initiation; several rats died during the hepatectomy procedure. All animals were killed after 8 weeks on study, the liver and kidney weights were measured, and the organ to body weight ratios were calculated. A color video image processor was used to determine the number and areas of γ-GT-positive foci.

No dose-related mortality was observed. There was no significant difference in body weight or feed consumption when the group 1 rats fed *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate were compared with the rats in group 2. The calculated intake of test compound for the group 1 rats fed 110, 330, and 1000 ppm *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate and 600 ppm 3′-Me-DAB was 9, 24, 76, and 41 mg/kg/day, respectively.

N,N-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate-related gross hepatic lesions were not observed. Relative kidney to body weight in group 1 rats fed 330 ppm *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate were significantly less than the value obtained for group 2 rats; no dose-dependency was observed.

The number and area of γ-GT-positive foci/cm^2 in rats fed *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate was not significantly different than the values obtained for group 2. A few γ-GT-positive foci of very small size were found in group 3 animals fed 1000 ppm *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate without initiation; no lesions without initiation were observed in any of the other *N,N-Bis*(2-

Hydroxyethyl)-*p*-Phenylenediamine Sulfate-fed groups. *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate at oral doses of ≤1000 ppm was not hepatocarcinogenic and did not act as a promoter of hepatic neoplasms in rats in this test.

CLINICAL ASSESSMENT OF SAFETY

Irritation/Sensitization

A repeated insult patch test (RIPT) was conducted to determine the irritation/sensitization potential of *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate.[20] All subjects were female; 116 subjects began and 104 completed the study. Occlusive patches were used to apply 0.1 ml of 3.0% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate in vehicle. The application site was the intrascapular area of the back, either to the right or left of the midline.

The initial patches were removed 24 h after application and the test site was evaluated 48 h after dosing. A new patch was applied after evaluation. Patches applied on a Friday were removed after 24 h, but not evaluated until 72 h after dosing. Nine doses were applied over 3 weeks.

Following test site evaluation of the ninth dose, there was a nontreatment period of 2 weeks. A challenge was then performed with an identical patch being applied to a previously untreated site. Patches were removed after 24 h, and the site was evaluated 48 and 72 h after test substance application.

One subject had definite erythema with minimal or doubtful edema 48 h after and definite erythema with no edema 72 h after challenge; this indicated probable irritation and possible sensitization. One subject had erythema with definite edema and vesiculation, with some spreading of the reaction beyond the test site, 48 and 72 h after the challenge application; this reaction was indicative of definite sensitization. A third subject had reactions during induction and challenge; this subject was thought to be pre-sensitized to the vehicle. *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate produced definite sensitization in one subject and possible sensitization in another.

Epidemiology

Forty percent of women in the United States are estimated to be regular users of hair dyes.[11] Under normal conditions of use, skin contact with the hair dye is restricted to 30 minutes. Professional hair dressers would be expected to incur greater exposure.

Much research has been undertaken in order to determine if there is a correlation between hair dye use and cancer. A number of studies were reviewed by the International Agency for Research on Cancer (IARC).[21,22] The IARC Working Group found that evidence linking various cancers to occupational or personal hair dye use was inconclusive.

SUMMARY

N,N-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate is an oxidative hair dye that is a derivative of *p*-Phenylenediamine and it occurs as light gray or pink powder or crystals. In 1984, it was reported to the FDA that *N,N-Bis*(2-Hydroxyethyl)-*p*-Phe-

nylenediamine Sulfate was being used in 183 hair dye and color formulations at concentrations ≤5%.

The oxidative or permanent hair dyes containing *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate, as oxidative 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. In order to be exempt, the following caution statement must be displayed on all oxidative hair dye products:

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

Patch test instructions call for a 24 h patch on the skin of the user with the intermediates and hydrogen peroxide mixed in the same manner as in use. This test is to be performed prior to each and every application of the hair dye.

N,N-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate has a minimum purity of 95%.

N,N-Bis(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate was moderately toxic to male rats according to the terminology of Hodge and Sterner;[23] the compound has an oral LD_{50} of 264 mg/kg.

Sprague-Dawley rats were fed ≤0.3, 0.5 or 0.75% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate in diet for 5, 2, or 2 weeks, respectively. Rats fed 0.5 or 0.75% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate had reduced body weights and feed consumption. At necropsy, the kidneys were discolored, some livers were enlarged, and certain organ weights were significantly different when compared with the controls.

Sprague-Dawley rats were fed diet containing ≤0.30% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate for 13 weeks. Body weights and feed consumption were decreased. *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate administration produced darkened thyroid glands, decreased serum iron concentrations, and reduced pituitary gland weights. *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate, ≤1.0%, was dermally applied to New Zealand White rabbits twice weekly for 13 weeks. No toxicologically significant results were observed.

Male and female Sprague-Dawley rats were fed ≤0.30% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate in the diet for a minimum of 26 weeks. A recovery group of male rats ate treated feed for a minimum of 20 weeks, and then untreated feed for 6–8 weeks. Some males and females had darkened thyroid glands. Increased kidney and heart weights relative to body weights (which were not thought to be toxicologically important), decreased hematocrit values, and increased serum phosphate concentrations were observed for some males.

In two sensitization studies using guinea pigs, 3% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate induced delayed hypersensitivity. *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate, 2%, produced a strong contact allergic, but not a photoallergic, response.

Neither teratogenic nor embryonic effects were produced in rats by dermal administration of ≤1.0% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate or oral

administration of ≤0.3% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate in the diet. A dominant lethal effect did not result from feeding male rats ≤0.3% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate in the diet.

A study investigating the potential promoting effects of *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate on hepatic cancer found that test compound fed to rats in the diet was not hepatocarcinogenic and did not act as a promoter of liver cancer.

A human repeated insult patch test using 3.0% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate, in which 104 of 116 female subjects completed the study, produced definite sensitization in one subject and possible sensitization in another.

DISCUSSION

The Expert Panel recognizes that 3% *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate has been shown to induce delayed hypersensitivity in guinea pigs. However, *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate is an oxidative hair dye ingredient and, therefore, 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 the following caution statement and "patch test" instructions for determining whether the product causes skin irritation:

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

Patch test instructions call for a 24 h patch on the skin of the user with the intermediates and hydrogen peroxide mixed in the same manner as in use. This test is to be performed prior to each and every application of the hair dye.

In making an assessment of safety, the Panel noted that *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate was not regarded as a hepatic carcinogen. Although no eye safety test data were available, the Expert Panel noted that the required product labeling specifically states that hair dye products should not be used to dye either the eye lashes or eyebrows.

CONCLUSION

On the basis of the animal and clinical data presented in this report, the CIR Expert Panel concludes that *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate is safe as a cosmetic ingredient in the present practices of use and concentration.

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REFERENCES

1. NIKITAKIS, J.M. (Editor). (1988). CFTA Cosmetic Ingredient Handbook. Washington, DC: CTFA, p. 131.

2. HAGIWARA, A., TAMANO, S., SHIBATA, M.-A., ARAI, M., and TSUDA, H. (1989). Lack of modifying effects of 2,2'-[(4-Aminophenyl)Imino] Bisethanol Sulfate on induction of preneoplastic γ-glutamyl transpeptidase-positive foci in a medium-term bioassay system using F344 rats. J. Toxiol. Sci. 14(2):77–86.
3. ELDER, R.L. (1985). Final report on the safety assessment of *p*-Phenylenediamine. J. Am. Coll. Toxicol. **4**(3), 203–66.
4. ESTRIN, N.F., CROSLEY, P.A., and HAYNES, C.R. (Editors). (1982). *CTFA Cosmetic Ingredient Dictionary*. Washington, DC: CTFA, pp. 28–9.
5. CLAIROL. (1985). Combined subchronic feeding, teratology, and dominant lethal study on *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate in Sprague-Dawley rats. (Study #84002). Unpublished data submitted to CTFA.[1]
6. COSMETIC, TOILETRY, AND FRAGRANCE ASSOCIATION (CTFA). (1991). Personal communication with CTFA.
7. BURNETT, C.M., RE, T.A., LOEHR, R.F., RODRIGUEZ, S.C., and CORBETT, J.F. (1986). Evaluation of the teratological and dominant lethal potential of *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate in a 6-month feeding study in rats. Fd. Chem. Toxic. **24**(8), 875–80.
8. FOOD AND DRUG ADMINISTRATION (FDA). (1984). Frequency of use data.
9. CODE OF FEDERAL REGULATIONS. (April 1, 1982). Title 21, Part 720.4. Voluntary filing of cosmetic product ingredient and cosmetic raw material composition statement. Information requested about cosmetic products.
10. FEDERAL REGISTER. (Oct. 16, 1979). Cosmetic Product Warning Statements; coal tar hair dyes containing 4-Methoxy-*m*-Phenylenediamine (2,4-diaminoanisole) or 4-Methoxy-*m*-Phenylenediamine Sulfate (2,4-diaminoanisole sulfate). 44(201): 59509-10.
11. CORBETT, J.F., and MENKART, J. (1973). Hair coloring. Cutis **12,** 190–7.
12. CLAIROL. (1984). Pilot feeding study on *N,N-Bis*(2-Hydroxyethyl)*p*pd Sulfate in the Sprague-Dawley rat. (Study #83/30/034). (Appendix I of combined subchronic feeding, teratology, and dominant lethal study on *N,N-Bis*(2-Hydroxyethyl)-*p*-Phenylenediamine Sulfate in Sprague-Dawley rats. (Study #84002).) Unpublished data submitted to CTFA.[1]
13. BURNETT, C., GOLDENTHAL, E.I., HARRIS, S.B., WAZETER, F.X., STRAUSBURG, J., KAPP, R., and VOELKER, R. (1976). Teratology and percutaneous toxicity studies on hair dyes. J. Toxicol. Environ. Health **1,** 1027–40.
14. CLAIROL. (1988). Evaluation of the skin sensitization potential of oxidation hair dyes in the albino guinea pig. (Study #88022). Unpublished data submitted to CTFA.[1]
15. CLAIROL. (1982). Evaluation of the skin sensitization potential of purified RM-119 in the albino guinea pig. (Study #81/12/028). Unpublished data submitted to the Cosmetic, Toiletry, and Fragrance Association (CTFA).[1]
16. CLAIROL. (1986). Photosensitization potential of *N,N-Bis*(2-Hydroxyethyl)-*PPD* Sulfate (*N,N,Bis*) in guinea pigs. (Study #86013). Unpublished data submitted to CTFA.[1]
17. ITO, N., TSUDA, H., TATEMATSU, M., et al. (1988). Enhancing effect of various hepatocarcinogens on induction of preneoplastic glutathione S-transferase placental form positive foci in rats—an approach for a new medium-term bioassay system. Carcinogenesis **9,** 387–94. (Secondary reference.)
18. TATEMATSU, M., SHIRAI, T., TSUDA, H., et al. (1977). Rapid production of hyperplastic liver nodules in rats treated with carcinogenic chemicals: A new approach for an in vivo short-term screening test for hepatocarcinogens. Gann, **68:**499–507. (Secondary reference).
19. TSUDA, H., HASEGAWA, R., IMAIDA, K., et al. (1984). Modifying potential of thirty-one chemicals on the short-term development of γ-glutamyl transpeptidase-positive foci in diethylnitrosoamine initiated rat liver. Gann **75,** 876–83. (Secondary reference.)
20. CLAIROL. (1984). Repeated insult patch test. (Study #841012). Unpublished data submitted to CTFA.[1]
21. IARC. (1982). IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. Vol. 16. Lyon, France: IARC, pp. 51–62.
22. IARC. (1987). IARC Monographs on the establishment of the risk of chemicals to humans. Overall evaluation of carcinogenicity and updating of IARC monographs. Vol. 1-42, Suppl. 7. Lyon, France: IARC, p. 61.
23. HODGE, H.C., and STERNER, J.H. (1949). Tabulation of toxicity classes. Am. Indus. Hyg. A. Quart. **10,** 93–6.

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

Final Report on the Safety Assessment of 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Chloro-2-Aminophenol[1]

Each of these ingredients function as hair colorants. 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol are identified as oxidative hair dyes, that is, they are combined with an oxidizing agent before being applied to the hair. 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, and 5-Amino-4-Chloro-*o*-Cresol are used in oxidative hair dyes, but it is not known if they are also used in nonoxidative (semipermanent) hair dyes. No toxicologically significant impurities are present with these two ingredients. To supplement the safety test data on these ingredients, available data on related ingredients (4-amino-2-hydroxytoluene and *p*-, *m*-, and *o*-aminophenol) previously found safe as used by the Cosmetic Ingredient Review (CIR) Expert Panel were summarized. 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol do not absorb significant ultraviolet radiation in the UVB region and none in the UVA region, although 4-Amino-*m*-Cresol had a symmetrical UV absorption peak at 300 nm. Percutaneous penetration of 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol alone was significant, but when combined with oxidative developer, skin absorption was extremely low. Both of these dyes are excreted rapidly via the urine. Repeated exposure of animal skin to 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol failed to produce any cumulative irritation and single exposures up to 10% were not irritating to animal skin. 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol combined with oxidizer were not sensitizers in guinea pig maximization tests. Ocular irritation resulted from exposure of animals to undiluted 5-Amino-4-Chloro-*o*-Cresol, but not to a 5% solution. Only minor irritation was observed with 5% 5-Amino-6-Chloro-*o*-Cresol. Subchronic toxicity testing in animals using 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Amino-*m*-Cresol did not yield any adverse reactions. 6-Amino-*m*-Cresol and 4-Amino-*m*-Cresol were generally not mutagenic in in vitro and in vivo tests. Exposure to 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, 6-Amino-*m*-Cresol and 4-Amino-*m*-Cresol from cosmetics were several orders of magnitude below developmental toxicity no-observed-adverse-effect levels (NOAELs). Although irritation data on several ingredients are absent, products containing these ingredients must include a caution statement and patch test instructions for determining whether the product causes skin irritation. The Expert Panel expects that following this procedure would identify individuals who would have an adverse reaction and allow them to avoid significant exposures. These compounds, when tested alone, are moderate skin sensitizers, but when combined with the developer, these ingredients are not sensitizers in animal tests. This information, coupled with the available animal test data, supports the safety of these ingredients in oxidative hair dyes. In the absence of systemic toxicity data, however, the available data are insufficient to support the safety of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol in semipermanent hair dyes. The types of data required for these two ingredients for this use include (1) physical and chemical properties, including the octanol/water partition coefficient; (2) impurities data, especially regarding the presence of *m*-cresol, other organic molecules, and heavy metals; (3) data demonstrating that the metabolism is similar to that of 4-amino-2-hydroxytoluene and/or *p*-, *m*-, and *o*-aminophenol, or 28-day dermal toxicity with histopathology, dermal reproductive toxicity data, and an in vitro genotoxicity study for 6-Amino-*o*-Cresol and one genotoxicity study in a mammalian system; if positive, a 2-year dermal carcinogenicity study using National Toxicology Program methods may be needed.

INTRODUCTION

This report reviews the safety of 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Chloro-2-Aminophenol, all of which function as hair colorants (Pepe, Wenninger, and McEwen 2002).

Data from the Cosmetic Ingredient Review (CIR) reports on 4-amino-2-hydroxytoluene and *p*-, *m*-, and *o*-aminophenol, and relevant data on other structurally similar ingredients (including the hepatotoxicity of acetaminophen derivatives), are included in this review. Elder (1989) found 4-amino-2-hydroxytoluene and Elder (1988) found *p*-, *m*-, and *o*-aminophenol safe in the present practices of use and concentrations. For purposes of comparison with the ingredients reviewed in this safety assessment, 4-Amino-2-hydroxytoluene was used in hair dyes and tints at concentrations $\leq$5% and *p*-, *m*-, and *o*-aminophenol were

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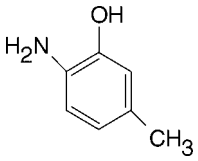
used in hair tints and hair dyes and colors at concentrations of ≤1%, ≤5%, and ≤1%, respectively.

CHEMISTRY

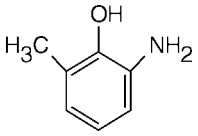
Definition and Structure

6-Amino-*m*-Cresol (CAS no. 2835-98-5) is the substituted aromatic compound that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



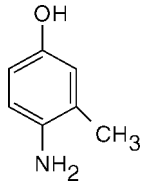
6-Amino-*m*-Cresol is also known as 4-Amino-3-Hydroxytoluene; 2-Amino-5-Methylphenol; Phenol, 2-Amino-5-Methyl-; 2-Hydroxy-4-Methylaniline (Pepe, Wenninger, and McEwen 2002); *m*-Cresol, 6-Amino; 6-Amino-3-Cresol; 6-Amino-3-Methylphenol; 2-Hydroxy-*p*-Toluidine; 5-Methyl-2-Aminophenol (Regulated Chemicals Listing 1998); 6-Amino-*meta*-Cresol; 4-Amino-3-Oxy-1-Methyl-Benzol; 4-Amino-3-Oxy-Toluol (Beilstein File of Organic Compounds 1998); and Toluene, 4-Amino-3-Hydroxy (CRC Handbook of Data on Organic Compounds 1998).

6-Amino-*o*-Cresol (CAS no. 17672-22-9) is the substituted aromatic compound that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



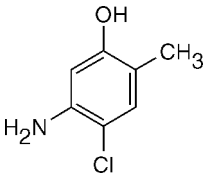
6-Amino-*o*-Cresol is also known as 3-Amino-2-Hydroxytoluene; 2-Amino-6-Methylphenol; Phenol, 2-Amino-6-Methyl-; 6-Amino-2-Methylphenol; Phenol, 6-Amino-2-Methyl-; 2-Hydroxy-3-Methylaniline (Pepe, Wenninger, and McEwen 2002); *o*-Cresol, 6-Amino; 6-Methyl-2-Aminophenol (Regulated Chemicals Listing 1998); 3-Amino-2-Oxy-1-Methylbenzol; and 3-Amino-2-Oxy-Toluol (Beilstein File of Organic Compounds 1998).

4-Amino-*m*-Cresol (CAS no. 2835-99-6) is the substituted aromatic compound that conforms to the formula (Pepe, Wenninger, and McEwen 2002):



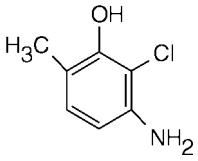
4-Amino-*m*-Cresol is also known as 2-Amino-5-Hydroxytoluene; 4-Amino-3-Methylphenol; Phenol, 4-Amino-3-Methyl-; 4-Hydroxy-*o*-Toluidine (Pepe, Wenninger, and McEwen 2002); 3-Methyl-4-Aminophenol (James Robinson Ltd. 1998); *p*-Amino-*m*-Cresol; *m*-Cresol, 4-Amino-; 4-Hydroxy-2-Methylaniline; *p*-Hydroxy-*o*-Toluidine; *m*-Methyl-*p*-Aminophenol; 3-Methyl-4-Aminophenol; 2-Methyl-4-Hydroxyaniline (Regulated Chemicals Listing 1998); 4-Amino-*meta*-Cresol; 6-Amino-3-Oxy-1-Methylbenzol; 6-Amino-3-Oxy-Toluol; *p*-Hydroxy-*o*-Toluidine; and Toluene, 2-Amino-5-Hydroxy (CRC Handbook of Data on Organic Compounds 1998).

5-Amino-4-Chloro-*o*-Cresol (CAS no. 110102-86-8) is an organic compound that conforms to the formula:



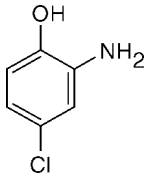
5-Amino-4-Chloro-*o*-Cresol is also known as 5-Amino-4-Chloro-2-Methylphenol; Phenol, 5-Amino-4-Chloro-2-Methyl- (Pepe, Wenninger, and McEwen 2002); and 2-Methyl-4-Chlor-5-Aminophenol (Henkel KGaA 1994).

5-Amino-6-Chloro-*o*-Cresol (CAS no. 84540-50-1) is an organic compound that conforms to the formula:



5-Amino-6-Chloro-*o*-Cresol is also known as 3-Amino-2-Chloro-6-Methylphenol; Phenol, 3-Amino-2-Chloro-6-Methyl- (Pepe, Wenninger, and McEwen 2002; Regulated Chemicals Listing 1998); 2-Chloro-3-Amino-6-Methylphenol; 2-Chloro-6-Methyl-3-Aminophenol; 3-Amino-2-Chlor-6-Methylphenol; 2-Methyl-5-Amino-6-Chlorophenol (Regulated Chemicals Listing 1998); 2-Hydroxy-3-Chloro-4-Aminotoluene; 2-Hydroxy-3-Chlor-4-Aminotoluol; and 5-Amino-6-Chlor-Benzol (Henkel KGaA 1996).

4-Chloro-2-Aminophenol (CAS no. 95-85-2) is the hair colorant that conforms to the formula:

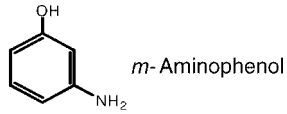


4-Chloro-2-Aminophenol is also known as 2-Amino-4-Chlorophenol; Phenol, 2-Amino-4-Chloro-; 2-Hydroxy-5-Chloroaniline; CI 76525 (Pepe, Wenninger, and McEwen 2002; Regulated Chemicals Listing 1998); 5-Chloro-2-Hydroxyaniline; *o*-Amino-*p*-Chlorophenol; *p*-Chloro-*o*-Aminophenol; and C.I. Oxidation Base 18 (Regulated Chemicals Listing 1998).

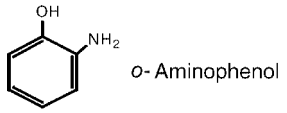
TABLE 1
Physical and chemical properties of 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol (Henkel KGaA 1994, 1996)

	Description	
Property	5-Amino-4-Chloro-*o*-Cresol	5-Amino-6-Chloro-*o*-Cresol
Form	Brown crystals	Beige crystals
Melting point	248°C (with decomposition)	144–183°C
Odor	None	None
Solubility	Soluble in water, propylene glycol, and triethanolamine	Soluble in water
Purity	97% (by HPLC)	>94% (by HPLC)
Molecular weight	157.59 (free base)	194.07 (hydrochloride)

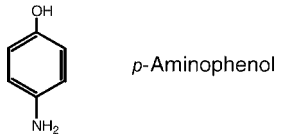
Structure of Related Ingredients



m-Aminophenol



o-Aminophenol



p-Aminophenol

The structures of *p*-, *m*-, and *o*-aminophenol are given above for comparison purposes. These ingredients were found safe in the present practices of use and concentrations (Elder 1988). Those use concentrations were ≤1%, ≤5%, and ≤1% for *p*-, *m*-, and *o*-aminophenol, respectively, in hair tints and hair dyes and colors.

Physical and Chemical Properties

6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, and 4-Amino-*m*-Cresol all have a molecular weight of 123.07 and 4-Chloro-2-Aminophenol has a molecular weight of 143.01 (Spectral Database Information System 1998). Other data on the physical and chemical properties of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol were not available. 6-Amino-*m*-Cresol (purum grade not defined) is a solid at room temperature (Goel, Kansal, and Sharma 1979). 4-Amino-*m*-Cresol has a melting point of 176°C to 178°C, is soluble in water and organic solvents, and a 1% solution had a pH of 8.2 (James Robinson Ltd. 1998).

Physical and chemical properties of 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol are shown in Table 1.

The melting point for 6-Amino-*m*-Cresol is 163°C (CTFA 1999a). It is slightly soluble in water and soluble in many organic solvents. It is 99.9% pure as determined by elemental analysis. 6-Amino-*m*-Cresol is a crystalline powder with a beige to reddish-brown color. Upon exposure to air it becomes darker. The ultraviolet (UV) absorption data for 6-Amino-*m*-Cresol indicated absorption maxima at 210, 235, and 291 nm in ethanol. Physical and chemical properties of 6-Amino-*m*-Cresol are listed in Table 2.

The melting point for 4-Amino-*m*-Cresol is 178°C (CTFA 1999b). It is slightly soluble in water and is a crystalline powder with a reddish-brown color. It is 99.9% pure as determined by elemental analysis. When heated to decomposition it emits toxic fumes of NO. 4-Amino-*m*-Cresol is stable at normal conditions and hazardous polymerization will not occur. According to the classification of the European Directive on Classification of Hazardous Preparations, 90/492/EEC, 4-Amino-*m*-Cresol is not

TABLE 2
Physical and chemical properties of 6-Amino-*m*-Cresol and 4-Amino-*m*-Cresol (CTFA 1999a, 1999b)

	Description	
Property	6-Amino-*m*-Cresol	4-Amino-*m*-Cresol
Form	Beige to reddish-brown crystals	Reddish-brown crystals
Melting point	163°C	178°C
Odor	Not available	Emits toxic fumes of NO when heated
Solubility	Slightly soluble in water, and many organic solvents	Slightly soluble in water
Purity	99.9% (by HPLC/GC)	99.9% (by HPLC/GC)
Molecular weight	123.16	123

a dangerous substance. The UV absorption data for 4-Amino-*m*-Cresol indicated absorption maxima at 206, 234, and 300 nm in ethanol. Physical and chemical properties of 4-Amino-*m*-Cresol are also listed in Table 2.

Manufacture and Production

Published data on the manufacture and production of 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, or 4-Chloro-2-Aminophenol were not found.

Analytical Methods

6-Amino-*m*-Cresol and 4-Amino-*m*-Cresol have each been separated using capillary electrophoresis and high-performance liquid chromatography (HPLC) utilizing crown ethers (Nishi et al. 1997). 4-Amino-*m*-Cresol has been determined using thin-layer chromatography, and identified in urine using HPLC (Son, Everett, and Fiala 1980).

Ultraviolet Absorbance

Published data on the UV absorbance of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol were not found. 6-Amino-*m*-Cresol has maximum absorption peaks at 210, 235, and 291 nm in ethanol (CTFA 1999a). 4-Amino-*m*-Cresol had a symmetrical absorption peak at 300 nm (James Robinson, Ltd. 1998) and maximum absorption peaks at 206, 234, and 300 nm in ethanol (CTFA 1999b).

5-Amino-4-Chloro-*o*-Cresol has a symmetrical absorption peak below 300 nm, which falls off sharply above 300 nm (Henkel KGaA 1994), and 5-Amino-6-Chloro-*o*-Cresol has a similar pattern with an even sharper fall off (Henkel KGaA 1996).

4-Amino-2-hydroxytoluene has a maximum UV absorbance at approximately 285 nm (Elder 1989).

Impurities

Published data on the impurities of 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, or 4-Chloro-2-Aminophenol were not found.

The impurity limits for 4-Amino-*m*-Cresol specify >99.5% solid content, <1.0% sulfated ash, and <50 ppm iron, with assay of >98.0% (James Robinson Ltd. 1998). The typical analysis was >99.9% solid content, <0.5% sulfated ash, and <10 ppm iron, with assay of 98.5% to 99.5%. No *m*-cresol was detected by HPLC.

The specification of 97% purity for 5-Amino-4-Chloro-*o*-Cresol is supported by HPLC analysis; impurities include an early peak identified as 2-Methyl-5-Aminophenol (2%), and two unidentified peaks (1% combined), one of which was close to the peak of the ingredient and one that eluted later (Henkel KGaA 1994).

An HPLC analysis of 5-Amino-6-Chloro-*o*-Cresol yielded 94.19% of the ingredient in one peak. Near the major peak were small peaks for 5-Amino-4-Chloro-2-Methylphenol (2.76%) and *p*-Amino-*o*-Cresol (1.99%). The only other significant peak (0.83%) was identified as a dichloro derivative (Henkel KGaA 1996).

USE

Cosmetic

6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Chloro-2-Aminophenol function as hair colorants (Pepe, Wenninger, and McEwen 2002).

5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol are specifically for use in oxidative hair dyes, with the former being used in combination with hydrogen peroxide (Henkel KGaA 1994, 1996).

The product formulation data submitted by the Food and Drug Administration (FDA) in 1998 stated that 6-Amino-*m*-Cresol was used in two hair dye and color formulations (FDA 1998). The other ingredients reviewed in this assessment were not reported to FDA as being used in 1998.

Concentration of use values are no longer reported to the FDA by the cosmetic industry (FDA 1992); the last reported concentration of use data available to CIR is from 1984 (FDA 1984). None of the ingredients reviewed in this report, however, were listed as being used in 1984.

Current information from industry indicated that 6-Amino-*m*-Cresol was used at a concentration of 2.4%, 6-Amino-*o*-Cresol was used at a concentration of 0.7%, and 4-Amino-*m*-Cresol was used at a concentration of 0.3% in all types of hair dye and colors (which require a caution statement and patch test) (CTFA 1999c).

In addition, 5-Amino-4-Chloro-*o*-Cresol is reported to be used in oxidation hair dye formulations at concentrations up to 2%, but because it is combined with hydrogen peroxide, the use concentration is only up to 1% (Henkel KGaA 1994). 5-Amino-6-Chloro-*o*-Cresol is also reported to be used in oxidative hair dyes formulations up to a final concentration of 2% (Henkel KGaA 1996).

Hair-coloring formulations are applied to or can come in contact with hair, skin (particularly at the scalp), eyes, and nails. Individuals dyeing their hair could use such formulations once every few weeks, whereas hairdressers could come in contact with products containing these ingredients several times a day. Under normal conditions of use, skin contact with hair dye is restricted to 30 min.

The hair dyes containing 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Chloro-2-Aminophenol, 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* of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin

irritation. The following caution statement should be displayed conspicuously on the labels of coal tar hair dyes:

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

The 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 h after application of the test material and prior to the use of a hair dye formulation.

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

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

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

6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Chloro-2-Aminophenol do not appear in Annex II (list of substances which must not form part of the composition of cosmetic products) or Annex III (list of substances which cosmetic products must not contain except subject to the restrictions and conditions laid down) of the *Cosmetics Directive of the European Union* (European Union 1995).

Noncosmetic

No uses for these ingredients other than in cosmetics were found.

GENERAL BIOLOGY

Absorption, Distribution, and Metabolism

*6-Amino-*m-*Cresol, 6-Amino-*o-*Cresol, and 4-Amino-*m-*Cresol*

Published data on the absorption, distribution, metabolism, and excretion of 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, or 4-Amino-*m*-Cresol were not found.

*5-Amino-4-Chloro-*o-*Cresol*

Skin absorption of radioactive (^{14}C) 5-Amino-4-Chloro-*o*-Cresol was studied using six female Sprague-Dawley rats (mean weight 189.5 g). A formulation containing the ingredient, with *p*-toluenediamine sulfate, basic fatty acid emulsion, propylene glycol, water, and ammonia, was diluted 1:1 with water to make a final test ingredient concentration of 1.85%. This formulation (0.2 g) was applied to an intact, clipped area of skin (9 cm^2) for 72 h under semiocclusive conditions. The concentration of ingredient on the skin was 0.41 mg/cm^2.

Feces and urine were monitored for 72 h, after which time the animals were sacrificed and adrenal glands, blood, brain, fat, bone, heart, kidneys, liver, lungs, muscle tissue, ovaries, spleen, thyroid glands, untreated skin, and the remaining carcass were analyzed. The mean skin absorption was 32.7%. 5-Amino-4-Chloro-*o*-Cresol was excreted via urine (92%) and feces (8%). The concentration in kidneys (0.003%) at 72 h was the greatest of any of the organ/tissue samples. The stratum corneum at the site of application, obtained by tape stripping, had 0.22% of the radioactivity (Henkel KGaA 1994).

A similar study was performed using the same strain of female rats of the same weight range except that the formulation was diluted 1:1 with a developer consisting of 6% hydrogen peroxide before application. After 30 min contact, the test material was rinsed off. Samples were taken as above. The skin absorption in this case was only 1.28%. Excretion via urine (91%) and feces (9%) accounted for all that was absorbed; the concentration in organs/tissues was at or near the detection limit of the ^{14}C. The stratum corneum had 0.2% of the radioactivity and the dermis, likewise, had 0.2% (Henkel KGaA 1994).

In a third study, the metabolism of ingested 5-Amino-4-Chloro-*o*-Cresol Hydrochloride was investigated using six female Sprague-Dawley rats (mean weight 200 g). A 1.27% solution of ^{14}C 5-Amino-4-Chloro-*o*-Cresol Hydrochloride in a 1:1 propylene glycol/water solution was given by oral administration at a dose of 21.5 mg/kg. Feces, urine, organs, and tissues were examined as described above. 5-Amino-4-Chloro-*o*-Cresol Hydrochloride was readily absorbed in the intestine (91.7%). It was excreted via urine (94%) and feces (6%). The greatest concentration in the organ/tissue samples was 0.001% in the liver (Henkel KGaA 1994).

*5-Amino-6-Chloro-*o-*Cresol*

Skin penetration/absorption of radioactive (^{14}C) hydrochloride was determined in a study using 12 female Wistar rats (mean weight 231 $\pm$ 7 g). Test animals were clipped and their skin

anesthetized with an i.m. injection of Ketanest® (12 ml/kg). In addition to the radioactive test ingredient, the formulation contained fatty alcohol, anionic surfactant, ammonium sulfate, water, and ammonia. The test article concentration was 1.14% and the pH was adjusted to 9.5. A dose of 20 mg/cm^2 was applied for 48 h without occlusive patches. Urine fractions were taken 0–8 h, 8–24 h, and 24–48 h. Feces were sampled daily. After 48 h, the animals were sacrificed and the skin and carcass assayed for radioactivity.

5-Amino-6-Chloro-*o*-Cresol hydrochloride was readily absorbed (93.2%). Radioactivity was excreted in urine (87.7%) and feces (2.22%). Only 0.48% was found in the carcass. The recovery rate of ^{14}C from the urine samples was 115% of the applied ^{14}C. An additional two animals were treated in the same manner, except that their expired CO_2 was monitored. No detectable ^{14}C was found in the expired CO_2 (Henkel KGaA 1996).

A similar study in six rats (mean body weight 217 ± 7 g) was conducted, except that the formulation was mixed 1:1 with 3% hydrogen peroxide developer solution prior to application. The test material was applied at a concentration of 15.3 mg/cm^2 and washed off after 30 min. Samples were collected as above. The skin penetration was only 0.116% (Henkel KGaA 1996).

The metabolism of radioactive (^{14}C) 5-Amino-6-Chloro-*o*-Cresol was determined in five female Wistar rats (weight 254 to 270 g). A single subcutaneous (s.c.) injection of 1 g of a 5-Amino-6-Chloro-*o*-Cresol solution (0.25% in water) was given into the neck. Urine, expired CO_2, and feces were collected over a period of 96 h. The animals were sacrificed and the skin and carcasses analyzed for residual radioactivity. Excretion was mainly via urine (88.5%) of which most (88.1%) was eliminated in the first 24 h. Only 3.97% was excreted in feces, and 0.674% was in the carcass and 0.04% in the injection site skin. No detectable radioactivity was found in expired CO_2 (Henkel KGaA 1996).

Metabolism was further studied using a single oral application of ^{14}C 5-Amino-6-Chloro-*o*-Cresol to 5 male Wistar rats (weight 321 to 336 g). Each animal received 49.4 mg/kg of the test article (1.7% in water) by gavage. Urine, expired CO_2, and feces were collected as daily fractions for 96 h. The animals were sacrificed and the gastrointestinal tract and the remaining carcass were analyzed. Excretion was again mainly via urine (90.93%) and mostly (90%) in the first 24 h. There was 6% in the gastrointestinal tract and 0.58% in the remaining carcass. No ^{14}C was detected in expired CO_2 (Henkel KGaA 1996).

The organ distribution of ^{14}C after a single oral dose of ^{14}C 5-Amino-6-Chloro-*o*-Cresol was studied in five male Wistar rats (mean weight 323 ± 9 g). A single dose of the test article (1.7% in water) was delivered by gavage. One rat was sacrificed at each of 1, 6, 24, 48, and 96 h after administration. Whole body autoradiography was used to detect the distribution of ^{14}C. Urine and feces were collected. One hour post administration the skin, kidneys, and the content of the intestine, liver, and especially the content of the stomach were collected for analysis. After 6 h, radioactivity was in the stomach, intestine, or colon content, and in the caecum. After 24 and 48 h, only residual radioactivity was found in the colon, caecum, and kidneys. After 96 h, excretion was nearly complete and only a small amount of label appeared (in bone). Within the first 24 h, 91% of the radioactivity was excreted via urine (Henkel KGaA 1996).

4-Amino-2-Hydroxytoluene and p-*Aminophenol*

Elder (1989) reported the percutaneous absorption of radioactive 4-amino-2-hydroxytoluene in a hair dye applied to the dry hair of humans under normal use conditions. The total excretion of 4-amino-2-hydroxytoluene was 0.2% ± 0.1%. This is contrasted with the oral administration in humans of radioactive 4-amino-2-hydroxytoluene in which there was a 94% recovery of the radioactivity in the urine. Elder (1988) reported the percutaneous absorption of 4-amino-2-hydroxytoluene (nonradioactive) coupled with radioactive *p*-aminophenol. The resultant ^{14}C-indamine was determined in rats under the conditions of oxidative hair dyeing. As much as 11% of the radioactivity introduced as ^{14}C-*p*-aminophenol was detected in the excreta, viscera, and skin of rats (Elder 1988); the penetration of *p*-aminophenol was similar when not coupled with 4-amino-2-hydroxytoluene. The ^{14}C-indamine formed during the oxidation did not substantially penetrate the cutaneous barrier.

Immunological Effects

4-Chloro-2-Aminophenol

The response of leukocytes from female guinea pigs treated with 4-Chloro-2-Aminophenol was evaluated using the leukocyte adherence inhibition (LAI) technique (Naniwa 1982). Both 4-Chloro-2-Aminophenol and *p*-aminophenol were conjugated with protein by similar condensation reactions. Significantly greater amounts of LAI were found for *p*-aminophenol–protein conjugates in the treated guinea pigs, indicating that 4-Chloro-2-Aminophenol–sensitized lymphocytes could not differentiate between 4-Chloro-2-Aminophenol–and *p*-aminophenol–protein conjugates. This suggested that cross-sensitization can occur with *p*-aminophenol.

Nephrotoxicity

4-Chloro-2-Aminophenol

Renal cortical slices from male Fischer 344 rats were used in gluconeogenesis and lactate dehydrogenase (LDH) release studies (Hong et al. 1996). The tissue slices were incubated with 0.01 to 0.5 mM 4-Chloro-2-Aminophenol in dimethyl sulfoxide (DMSO), 4-amino-2-chlorophenol, or vehicle. Renal gluconeogenesis was inhibited by ≥0.01 mM 4-Chloro-2-Aminophenol and ≥0.05 mM 4-amino-2-chlorophenol. LDH leakage was increased at concentrations of ≥0.5 mM 4-Chloro-2-Aminophenol and ≥0.1 mM 4-amino-2-chlorophenol.

p-*Aminophenol*

Hong et al. (1996), in an introduction to their study of chloro amino phenols, characterized *p*-Aminophenol as an acute

nephrotoxicant and a mild hepatotoxicant; *o*-Aminophenol as not toxic to the kidney or liver; and neither 4-Amino-3-chlorophenol nor 2-amino-5-chlorophenol as marked nephrotoxicant(s).

Hepatotoxicity

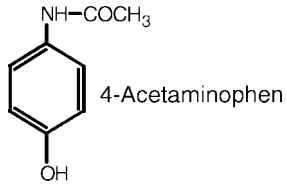
No data were available on ingredients in this safety assessment, but data on related ingredients are summarized below.

p-*Aminophenol and* o-*Aminophenol*

Elder (1988) reported that *p*-Aminophenol induces mild hepatotoxicity characterized by a twofold increase in serum transaminase levels, but that *o*-Aminophenol has no toxic effects on kidney or liver.

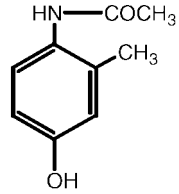
Acetaminophen

Acetaminophen, structure shown below, is somewhat similar to ingredients considered in this report and can be hepatotoxic in humans and experimental animals at large doses (Harvison, Forte, and Nelson 1986).

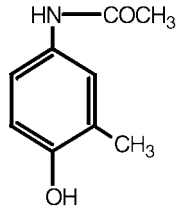


4-Acetaminophen

In a study to examine the role of mono-methylation in both the analgesic effect and hepatotoxicity of acetaminophen, Harvison, Forte, and Nelson (1986) prepared the following analogues that are structurally very similar to ingredients in this report:



4-Acetamino-*m*-Cresol



4-Acetamino-*o*-Cresol

Male Swiss-Webster mice (20 g) were injected intraperitoneally (i.p.) with either acetaminophen or the analogues shown above at various doses from 400 to 1000 mg/kg. Animals had been pretreated with either phenobarbital or cobaltous chloride and received a single i.p. dose of piperonyl butoxide 30 min before receiving the test substances. Animals were sacrificed and liver and kidney samples were taken and fixed in buffered formalin. Paraffin sections were prepared and stained with hematoxylin and eosin and examined for severity of necrosis.

The hepatotoxicity of 4-Acetamino-*o*-Cresol was comparable to that seen with acetaminophen, but 4-Acetamino-*m*-Cresol was less hepatotoxic. To the extent that these acetamino cresols are predictive of the hepatotoxicity of amino cresols, the results of these studies indicate that no greater hepatotoxicity would likely occur with the hair dye than is seen with acetaminophen, which isn't seen until g/kg doses are reached (Fethke, personal communication[2]).

ANIMAL TOXICOLOGY

Published data on the toxicity of 6-Amino-*o*-Cresol in animals was not found.

Acute Intraperitoneal Toxicity

4-Chloro-2-Aminophenol

Four male Fischer 344 rats per group were given a single i.p. injection of 0.4, 0.8, or 1.2 mmol/kg 4-Chloro-2-Aminophenol hydrochloride in 50% DMSO in distilled water, 0.4, 0.8, or 1.0 mmol/kg 4-amino-2-chlorophenol hydrochloride in distilled water, or vehicle (Hong et al. 1996). The animals were killed 48 h after dosing. 4-Chloro-2-Aminophenol had very few effects on renal function; no apparent morphological damage was observed at nonlethal doses of <0.8 mmol/kg. Changes in hepatic function or morphology were not observed. A dose of 1.2 mmol/kg 4-Chloro-2-Aminophenol killed 75% of the animals, but little evidence of nephrotoxicity was observed in the surviving animals. However, 4-amino-2-chlorophenol induced marked changes in renal function and morphology in a dose-dependent manner; no effect on hepatic function or hepatic morphology was observed.

Acute Dermal Toxicity

4-Amino-2-Hydroxytoluene

In an acute dermal toxicity study, 4-amino-2-hydroxytoluene did not produce any systemic/dermal toxicity in rabbits at a dose of 5 g/kg (Elder 1989).

p-*Aminophenol*

The dermal LD_{50} of *p*-aminophenol was >8 g/kg for rabbits (Elder 1988).

Acute Oral Toxicity

5-Amino-4-Chloro-o-*Cresol*

Male and female Wistar rats (average body weight of 164 g for females and 183 g for males) were given 5-Amino-4-Chloro-*o*-Cresol hydrochloride by gavage at doses of 1184, 1539, and 2000 mg/kg. Observations included apathy, piloerection, cyanosis, tremor, crouch, diarrhea, semiclosed eyes, and impaired hearing. Gross observations included brightened coloration of the liver and kidneys, ulcerations in the glandular

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

stomach, hydrometra, brown-colored hydrocele in the intestine, and emphysema (in the one animal that died). For males, the LD_{50} was between 1.54 and 2.0 g/kg and for females, the LD_{50} was >2.0 g/kg (Henkel KGaA 1994).

*5-Amino-6-Chloro-*o*-Cresol*

Male albino TNO-Wistar rats (average body weight of 200 g) were given 5-Amino-6-Chloro-*o*-Cresol hydrochloride by gavage at doses of 501, 1000, 1250, 1580, and 1999 mg/kg. Observations included apathy, staggering, rapid breathing, dyspnea (at later stages), and yellow-orange discoloration of the urine. The LD_{50} was 1.36 g/kg (Henkel KGaA 1996).

*4-Amino-*m*-Cresol*

Male CD-1 mice were dosed for 2 consecutive days (6 mice/group, route of administration not specified) with 1000, 1200, 1440, 1728, or 2074 mg/kg 4-Amino-*m*-Cresol. At 4 hours through day 2 of dosing, the following observations were observed: piloerection was observed in all groups; hypokinesia was observed in all but the low-dose group; ataxia occurred in the 1440- and 2074-mg/kg dose groups; and only mice in the 1200-mg/kg dose group had prostration. At least one mouse in all groups survived until day 14, but most mice died on day 1 or 2. The LD_{50} value was calculated as 1000 mg/kg (Holmstroem 1980).

*6-Amino-*m*-Cresol*

Holmstroem (1980), using the same protocol described above, calculated the LD_{50} of 6-Amino-*m*-Cresol as 1500 mg/kg.

In a pre-experiment toxicity study, Völkner and Heidemann (1991) dosed NMRI mice (2/sex/group) once with 500, 750, 1000, and 1500 mg/kg 6-Amino-*m*-Cresol in polyethylene glycol 400. Toxic reactions were observed in all groups and included reduction of spontaneous activity, eyelid closure, abdominal position, tremor, and death. One death occurred in each of the 750-, 1000-, and 1500-mg/kg groups by 6 h posttreatment. No deaths occurred in the 500-mg/kg group and the only toxic reaction observed in this group was reduction of spontaneous activity. Therefore, the 500-mg/kg group was estimated to be the maximum tolerated dose.

Leimbeck and Grötsch (1991) dosed two male and two female mice orally with 666 mg/kg 6-Amino-*m*-Cresol. In the first two hours all animals had tremor, anemia, and a slight to moderate reduction in activity. No animals died 72 h post application.

Fautz (1994) dosed two male rats once orally with 1200 mg/kg 6-Amino-*m*-Cresol in 1% carboxymethylcellulose. The rats had reduction of spontaneous activity, abdominal position, eyelid closure, and piloerection. In another experiment, two male rats each received a single oral dose of 1500 or 2000 mg/kg 6-Amino-*m*-Cresol in 1% carboxymethylcellulose, respectively. The animals in the 1500 mg/kg group had no toxic reactions except brown-colored urine. One animal in the 2000-mg/kg group died 24 h after treatment. The 1500-mg/kg group was estimated to be the maximum tolerated dose.

4-Amino-2-Hydroxytoluene

Using rats, 10% to 20% 4-amino-2-hydroxytoluene was slightly toxic in three separate acute oral studies (Elder 1989).

m-*Aminophenol,* o-*Aminophenol, and* p-*Aminophenol*

The oral LD_{50} values for rats of *p*-, *m*-, and *o*-aminophenol were 671–1270, 812–1660, and 1300 mg/kg, respectively (Elder 1988).

Short-Term Oral Toxicity

*6-Amino-*m*-Cresol*

Male and female Wistar rats (15/sex/group) were dosed orally with 50, 250, and 500 mg/kg 6-Amino-*m*-Cresol daily for 4 weeks (Forschungs GmbH 1985). The control group was dosed with 1 ml/100 g body weight 0.5% carboxymethylcellulose (CMC). Prior to study initiation and after 4 weeks, 10 rats/sex/group had ophthalmological and reflex examinations (5/sex/group), hearing tests and blood tests.

No significant observations occurred in the 50-mg/kg group. The 250-mg/kg group had increased activity 10 min after dosing during the third and fourth week of treatment and increased, discolored urine excretion. Water consumption was also increased. Significant results included reduced erythrocyte counts in males (highly significant) and females; increased reticulocytes in females; decreased hemoglobin in males and a highly significant decrease in females; increased hematocrit in both sexes, but highly significant in males; decreased iron in females; increased hepatic weight in females; increased kidney weight in males and females; and increased spleen weights in both sexes, but highly significant in females.

The 500-mg/kg group had initial decreased activity during week 1 and later increased activity as in the previous group. Increased, discolored urine excretion was also observed. Borderline significant results were observed for decreased body weight gain and food consumption during weeks 1 and 2 in females. Highly significant results were reported for increased water consumption in both sexes at all phases of the study; decreased erythrocytes and hemoglobin and increased reticulocytes in both sexes; and decreased hematocrit in males and females, although females were within normal range. The mean corpuscular volume (MCV) and prothrombin time was significantly increased in females, but still in the normal range. Iron was significantly reduced in females. At necropsy, dark, discolored spleens were observed (sex not specified). Liver, kidney, and spleen weights were all increased in both sexes. No treatment related observations were observed at microscopic evaluation. The no-observed-adverse-effect level (NOAEL) for 6-Amino-*m*-Cresol was established at 50 mg/kg.

Subchronic Dermal Toxicity

m-*Aminophenol,* o-*Aminophenol, and* p-*Aminophenol*

The dermal toxicity of hair dyes containing *m*-, *o*-, and/or *p*-aminophenol was determined using New Zealand white rabbits (Burnett et al. 1976). A dose of 1 ml/kg of oxidative hair dyes containing 0.7% *m*-aminophenol and 1.0% *p*-aminophenol, 0.7% *m*-aminophenol, 0.3% *o*-aminophenol, or 1.0% *N*-methyl-*p*-aminophenol sulfate mixed with an equal volume of 6% hydrogen peroxide or semipermanent hairdyes containing 0.09% and 0.2% *m*-aminophenol and p-aminophenol, respectively, or 0.02%, 0.04%, and 0.05% *m*-aminophenol, *p*-aminophenol, and *N*-methyl-*p*-aminophenol, respectively, were applied topically to the intact or abraded skin on the shaved backs of each animal twice weekly for 13 weeks, and no evidence of systemic toxicity was observed after application of the hairdyes.

Subchronic Oral Toxicity

5-Amino-4-Chloro-o-*Cresol*

Male and female Sprague Dawley rats (males, 152 to 160 g; females, 128 to 135 g) were given 5-Amino-4-Chloro-*o*-Cresol hydrochloride by gavage daily, 5 days a week, for 90 days. Daily doses were 0, 20, 60, and 180 mg/kg. No clinical observations or pathological findings indicative of systemic toxicity were observed. Only minor deviations in a few biochemical and hematological parameters were noted. The NOAEL was established at the highest dose of 180 mg/kg (Henkel KGaA 1994).

5-Amino-6-Chloro-o-*Cresol*

Male and female Wistar rats (males, 102 to 149 g; females, 98 to 138 g) were given 5-Amino-6-Chloro-*o*-Cresol hydrochloride with tragacanth (1%) by gavage daily, 5 days a week, for 13 weeks. Daily doses were 50 mg/kg. No clinical observations, biochemical alterations, or pathological findings were indicative of systemic toxicity. The NOAEL was established at the highest dose of 50 mg/kg (Henkel KGaA 1996).

4-Amino-m-*Cresol*

Male and female Wistar rats were dosed orally with 15, 60, or 120 mg/kg 4-Amino-*m*-Cresol for 13 weeks (Forschungs GmbH 1984a). A control group was also included. The control group and the 120-mg/kg group had 25 rats/sex/group and the low- and mid-dose groups had 20 rats/sex/group. Prior to study initiation and again at 6 and 13 weeks, 5 rats/sex/group had ophthalmological, hearing, and reflex examinations. Blood samples were taken at the same time intervals on 20 rats/sex/group. Urinalyses were performed on 5 rats/sex/group.

No specific observations occurred in the 15-mg/kg group. The 60- and 120-mg/kg groups had dark, discolored urine due to compound discoloration in both sexes from treatment weeks 8 to 13. The 120-mg/kg group had significantly increased creatinine values in the female rats after 13 weeks of treatment, although the values were still within the normal range. The spleen weights were significant in female rats and increased in male rats. No observations attributed to the test compound were found during microscopic evaluation. The NOAEL was established at the mid-dose, 60 mg/kg.

4-Amino-2-Hydroxytoluene

Elder (1989) reported that the administration of 4-amino-2-hydroxytoluene in the diet of rats at concentrations of $\leq$3% for 3 to 6 months caused reduction in body weight, a slight anemia, and sporadic microfollicular goiter. Feeding rats $\leq$0.7% *p*-aminophenol for 3 to 6 months resulted in decreased body weights and feed consumption, increased relative liver and kidney weights, and nephrosis. Feeding rats $\leq$1% *m*-aminophenol for 90 days resulted in decreased body weights and feed consumption, deposition of iron positive pigment in the spleen, liver, and kidneys, and increased thyroid gland activity.

Acute Dermal Irritation

6-Amino-m-*Cresol, 6-Amino*-o-*Cresol, 4-Amino*-m-*Cresol, and 4-Chloro-2-Aminophenol*

Published data on the dermal irritation potential of 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, or 4-Chloro-2-Aminophenol were not found.

5-Amino-4-Chloro-o-*Cresol*

The acute dermal toxicity of 5-Amino-4-Chloro-*o*-Cresol was determined using 3 adult female albino New Zealand white (SPF) rabbits. A 0.5-ml aliquot of 5-Amino-4-Chloro-*o*-Cresol was applied to intact, shaved skin on the dorsal back of each animal. A semiocclusive patch was applied. After 4 h the patch was removed and the site rinsed. The skin was examined immediately after patch removal and then at 1, 24, 48, and 72 h thereafter. Only very slight erythema and edema were seen at 24 h, which disappeared at 48 and 72 h. Brown-yellow/yellow staining was seen at the application site. No information on systemic toxicity was provided (Henkel KGaA 1994).

The acute dermal irritation of 5-Amino-4-Chloro-*o*-Cresol was determined using six adult male albino New Zealand rabbits. A 0.5-ml aliquot of a 10% formulation (3 g of 5-Amino-4-Chloro-*o*-Cresol, 10 ml of distilled water, and 5 ml ammonium sulfate dissolved to a total volume of 30 ml in 96% ethanol) was applied to intact, shaved skin on the dorsal back of each animal. An occlusive patch was applied for 2 h. The skin was examined immediately after patch removal and then at 24 and 48 h. No signs of erythema, edema, or eschar formation were seen and the animals had no signs of systemic toxicity (Henkel KGaA 1994).

5-Amino-6-Chloro-o-*Cresol*

The acute dermal toxicity of 5-Amino-6-Chloro-*o*-Cresol was determined using six adult male albino New Zealand rabbits. A 10% aqueous formulation (3 g of 5-Amino-6-Chloro-*o*-Cresol, 10 ml distilled water, and 5 ml ammonium sulfate dissolved to a total volume of 30 ml in 96% ethanol) was applied to a shaved area (0.5 ml/10 cm^2) on the dorsal back of each

animal. An occlusive patch was applied for 2 h. The skin was examined immediately after patch removal and then at 24 and 48 h. No signs of erythema, edema, or eschar formation were seen and the animals had no signs of systemic toxicity (Henkel KGaA 1996).

Repeated Dermal Application

5-Amino-4-Chloro-o-*Cresol*

Five adult male hairless mice (hr/hr strain) were used to assess skin irritation associated with repeated application of a 10% dilution of 5-Amino-4-Chloro-*o*-Cresol hydrochloride, adjusted to pH 8 with ammonia. Applications (one or two drops only) were made to the same area of the back once a day for 5 working days and twice a day for 4 working days for a total of 9 consecutive working days. Animals were examined before each application and the responses scored. No primary skin irritation was observed (Henkel KGaA 1994).

5-Amino-6-Chloro-o-*Cresol*

Five adult male hairless mice (hr/hr strain) were used to assess skin irritation associated with repeated application of a 10% aqueous formulation (3 g of 5-Amino-6-Chloro-*o*-Cresol, 10 ml distilled water, and 5 ml ammonium sulfate dissolved to a total volume of 30 ml in 96% ethanol). One drop was applied to the same spot on the dorsal back, twice per day, for 5 consecutive days. No signs of primary skin irritation were observed (Henkel KGaA 1996).

Repeated application of 5-Amino-6-Chloro-*o*-Cresol to 6 adult male New Zealand rabbits was studied by Henkel KGaA (1996). One drop of a 10% aqueous formulation (3 g of 5-Amino-6-Chloro-*o*-Cresol, 10 ml distilled water, and 5 ml ammonium sulfate dissolved to a total volume of 30 ml in 96% ethanol) was applied to the same shaved area of the dorsal back every 30 s for a total of 60 applications. No signs of primary irritation were observed.

4-Amino-2-Hydroxytoluene

Elder (1989) reported that a concentration of 2.5%, 4-amino-2-hydroxytoluene was essentially nonirritating.

m-*Aminophenol,* o-*Aminophenol, and* p-*Aminophenol*

Elder (1988) reported that *p*- and *m*-Aminophenol were mildly irritating to rabbit skin; that *p*- and *o*-Aminophenol were both nonirritating when applied to intact and abraded rabbit skin under occlusive patches and to intact rabbit skin under semiocclusive patches; and that *m*-Aminophenol, 3%, was not irritating when applied to the backs of rabbits.

Sensitization

6-Amino-m-*Cresol, 6-Amino*-o-*Cresol, and 4-Amino*-m-*Cresol*

Published data on the sensitization potential of 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, or 4-Amino-*m*-Cresol were not found.

4-Chloro-2-Aminophenol

The sensitization potential of 4-Chloro-2-Aminophenol and cross-sensitization potential with *p*-aminophenol was determined using guinea pigs (Naniwa 1982). (4-Chloro-2-Aminophenol and *p*-aminophenol belong to the same amino derivative class and have common side chains on the benzoic ring.) Fifteen female guinea pigs were first injected with an emulsion of 200 mg of 4-Chloro-2-Aminophenol in 0.5 ml *N*,*N*-dimethylformamide and 0.5 ml Freund's complete adjuvant. At 2 or 3, 4, and 6 weeks after treatment, the animals were patch tested with 0.1%, 0.5%, and 1.0% 4-Chloro-2-Aminophenol in equal volumes of dioxan and acetone. The solutions, 0.05 ml, were applied to the shaved dorsal area of each animal, and the sites were not covered. The test sites were scored 24 h after application of 4-Chloro-2-Aminophenol. Following patch testing with 4-Chloro-2-Aminophenol, a 1.0% *p*-aminophenol solution was applied using the same procedure. Five animals that were not treated were patch tested with 4-Chloro-2-Aminophenol and *p*-aminophenol and served as a control group.

One test animal died by week 6 of the study (reason for death not stated.) At weeks 2 to 3, 1, 1, and 3 of the 15 test animals had reactions (weak or strong erythema) at the 0.1%, 0.5%, and 1.0% 4-Chloro-2-Aminophenol sites, respectively. During the fourth week of the study, 2, 8, and 13 animals had reactions at the 0.1%, 0.5%, and 1.0% 4-Chloro-2-Aminophenol sites, respectively. During the sixth week of the study, 2, 7, and 13 of the 14 remaining test animals had reactions at the 0.1%, 0.5%, and 1.0% 4-Chloro-2-Aminophenol sites, respectively. None of the test animals reacted to *p*-aminophenol and none of the control animals reacted to 4-Chloro-2-Aminophenol or *p*-aminophenol.

5-Amino-4-Chloro-o-*Cresol*

Henkel KGaA (1994) conducted a guinea pig maximization study of 5-Amino-4-Chloro-*o*-Cresol using 20 female Pirbright White animals. Fifteen animals were used to determine the minimum irritant and maximum nonirritant concentration. Induction was done with injection of 0.1 ml of a 0.25% aqueous solution of 5-Amino-4-Chloro-*o*-Cresol (adjusted to pH 8 with ammonia) as the minimum irritant concentration and two injections of 0.1 ml of a 0.5% aqueous solution of 5-Amino-4-Chloro-*o*-Cresol diluted 1:1 with Freund's complete adjuvant (FCA). Controls were treated with FCA and vehicle only. The second topical induction was done 1 week later with 1.0 ml of a 5% aqueous solution of 5-Amino-4-Chloro-*o*-Cresol under an occlusive patch for 48 h. The challenge was done 14 days after the second induction with 0.2 ml of a 2% aqueous solution of 5-Amino-4-Chloro-*o*-Cresol applied to the animals' flanks under an occlusive patch. Animals were examined at 24 and 48 h after removal of the patch.

After the first and second inductions, all animals had typical reactions to FCA. Almost 50% of the test animals (9/19; no explanation provided for the fate of the 20th animal) had slight erythema 24 h after challenge, but only 5 animals had

this minimal effect after 48 h. It was concluded that 5-Amino-4-Chloro-*o*-Cresol is a moderate sensitizer in the maximization test.

Henkel KGaA (1994) performed a second maximization study using a hair dye formulation containing *p*-toluidine diamine and 5-Amino-4-Chloro-*o*-Cresol hydrochloride. The hair dye formulation was diluted 1:1 with 6% hydrogen peroxide before use in the experiment. As in the previous study, 15 female Pirbright White guinea pigs were used to determine irritant concentrations and 20 animals were included in the maximization test. Intradermal induction was done with injection of 0.1 ml of a 0.1% aqueous solution of the hair dye/oxidizer combination and two injections of a 0.2% solution diluted 1:1 with FCA. Controls were treated only with FCA and vehicle. The second, topical induction was done 1 week later with 1.0 ml of the test substance (hair dye/oxidizer combination) under an occlusive patch for 48 h. The challenge was done 14 days after the second induction using 0.2 ml of a 2.5% aqueous solution of the test material on the flank under an occlusive patch for 24 hours.

After the inductions, animals had typical reactions to FCA. None of the animals exposed to the test substance had any reactions. As found in a hair dye formulation mixed with an oxidizer, 5-Amino-4-Chloro-*o*-Cresol was a non-sensitizer in the maximization test.

Henkel KGaA (1994) conducted a third maximization test with a second hair dye formulation containing 2,4,5,6-tetraamino-pyrimidine and 5-Amino-4-Chloro-*o*-Cresol. The hair dye formulation was diluted 1:1 with 6% hydrogen peroxide as an oxidizer before use in the experiment. As above, 15 female Pirbright White guinea pigs were used to determine irritant concentrations and 20 animals were included in the maximization test. Intradermal induction was done with injection of 0.1 ml of a 0.1% aqueous solution of the hair dye/oxidizer combination and two injections of a 0.2% solution diluted 1:1 with FCA. Controls were treated only with FCA and vehicle. The second, topical induction was done 1 week later with 1.0 ml of a 20% aqueous solution of the test substance (hair dye/oxidizer combination) under an occlusive patch for 48 h. The challenge was done 14 days after the second induction using 0.2 ml of a 2.5% aqueous solution of the test material on the flank under occlusive patches for 24 hours.

After the inductions, animals had typical reactions to FCA. None of the animals exposed to the test substance had any reactions. As found in this second hair dye formulation mixed with an oxidizer, 5-Amino-4-Chloro-*o*-Cresol hydrochloride was a nonsensitizer in the maximization test (Henkel KGaA, 1994).

Henkel KGaA (1994) also performed a Buehler method sensitization test using Dunkin-Hartley guinea pigs. Four animals were used to determine minimum irritant and maximum nonirritant concentrations and 20 animals were used in the sensitization test proper. Topical induction was done on the left body side on days 1, 8, and 15 with 0.5 ml of an ethanolic paste consisting of 5-Amino-4-Chloro-*o*-Cresol in ethanol (63% w/w) under occlusive patches for 6 h. Control animals were dosed with ethanol only. The challenge was done 14 days later by exposing the animals' flanks to 0.5 ml of the paste for 6 h under occlusive patches. Animals were examined 24 and 48 h after patch removal.

Neither test animals nor controls had reactions on challenge, so 5-Amino-4-Chloro-*o*-Cresol was not considered to be a sensitizer in this test (Henkel KGaA 1994).

*5-Amino-6-Chloro-*o*-Cresol*

Henkel KGaA (1996) conducted a guinea pig maximization study of 5-Amino-6-Chloro-*o*-Cresol hydrochloride using 20 female Pirbright White animals. Induction was done with injection of 0.1 ml of a 5.0% aqueous solution of 5-Amino-6-Chloro-*o*-Cresol and two injections of 0.1 ml of a 5.0% aqueous solution of 5-Amino-6-Chloro-*o*-Cresol diluted 1:1 with FCA. Controls were treated with FCA and vehicle only. The second topical induction was done 1 week later with 1.0 ml of a 5% cream of 5-Amino-6-Chloro-*o*-Cresol in petroleum jelly under an occlusive patch for 48 h. The challenge was done 14 days after the second induction with a 25% cream of the test substance applied to the animals' flanks under an occlusive patch. Animals were examined at 24 and 48 h after removal of the patch.

After the first and second inductions, all animals had typical reactions to FCA. One quarter of the test animals had slight erythema 24 h after challenge, but no effects were evident after 48 h. It was concluded that 5-Amino-6-Chloro-*o*-Cresol is not a sensitizer in the maximization test (Henkel KGaA 1996).

Using guinea pigs, 4-amino-2-hydroxytoluene was a mild sensitizer in a maximization test and a very weak sensitizer in a test using an open epicutaneous method (Elder 1989). Application to guinea pigs of 0.1% to 2% *p*-aminophenol in petrolatum under occlusive patches resulted in a concentration-dependent incidence of sensitization, with 3 of 10 animals sensitized with 0.1% and 9 of 10 animals sensitized at 2% *p*-aminophenol (Elder 1988). *p*-Aminophenol, 3% in deionized water, was not a sensitizer in guinea pigs. In an open epicutaneous test using guinea pigs, 3% *p*-aminophenol produced weak reactions in 4 of 20 animals and 3% *m*-aminophenol was not a sensitizer. In a maximization test, moderately strong cross-reactions to *o*-aminophenol application were observed in some guinea pigs previously sensitized with *p*-phenylenediamine.

Photosensitization

Published data on the photosensitization potential of ingredients reviewed in this safety assessment were not found.

4-Amino-2-Hydroxytoluene

Elder (1989) reported that 4-Amino-2-hydroxytoluene, with induction and challenge concentrations of 5% and 10%, respectively, was not a photosensitizer when evaluated using guinea pigs.

m-*Aminophenol,* o-*Aminophenol, and* p-*Aminophenol*

Elder (1988) reported that *p*-Aminophenol and *m*-aminophenol, both with induction and challenge concentrations of 10% and 5%, respectively, were not photosensitizers, but they did induce a contact hypersensitivity reaction.

Ocular Irritation

6-Amino-m-*Cresol, 6-Amino*-o-*Cresol, 4-Amino*-m-*Cresol, and 4-Chloro-2-Aminophenol*

Published data on the ocular irritation potential of 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, or 4-Chloro-2-Aminophenol were not found.

5-Amino-4-Chloro-o-*Cresol*

A volume of 0.1 ml of 5% aqueous 5-Amino-4-Chloro-*o*-Cresol hydrochloride was instilled into the conjunctival sac of six male albino New Zealand rabbits; no rinsing was done. Eye irritation reactions were scored 2, 6, 24, and 48 h after exposure. No effects on the cornea or the iris, and only slight conjunctival erythema and edema up to 24 h were observed (Henkel KGaA 1994).

5-Amino-6-Chloro-o-*Cresol*

A quantity of 51 mg of 5-Amino-6-Chloro-*o*-Cresol hydrochloride was instilled into the conjunctival sac of the right eye of one female albino New Zealand rabbit; none of the eyes were rinsed. Ocular irritation reactions were scored 1, 24, 48, and 72 h after exposure. Instillation of the undiluted ingredient produced immediate severe ocular irritation, and additional study was terminated. Corneal opacity, injection of the iris, and irritation of the conjunctivae persisted throughout the duration of the study. Undiluted 5-Amino-6-Chloro-*o*-Cresol hydrochloride was considered a severe ocular irritant (Henkel KGaA 1996).

In a second study, a volume of 0.1 ml of 5% aqueous 5-Amino-6-Chloro-*o*-Cresol hydrochloride was instilled into the conjunctival sac of four male albino New Zealand rabbits; none of the eyes were rinsed. Ocular irritation reactions were scored 1, 6, 24, and 48 h after exposure. No effects on the cornea or the iris, and only slight conjunctival erythema up to 6 h were observed. Exudation was observed after 1 h in all four animals, in three animals at 6 h, and in one animal at 24 h; the effect was not seen at 48 h. The researchers considered 5% 5-Amino-6-Chloro-*o*-Cresol hydrochloride to be very slightly irritating (Henkel KGaA 1994).

4-Amino-2-Hydroxytoluene, m-*Aminophenol,* o-*Aminophenol, and* p-*Aminophenol*

At a concentration of 2.5%, 4-amino-2-hydroxytoluene (Elder 1989), *p*-aminophenol, and *m*-aminophenol (Elder 1988) were essentially nonirritating to rabbit eyes. In Draize tests, *p*-aminophenol (powder form) was not an eye irritant and *o*-Aminophenol did not irritate the cornea or iris and produced a cumulative conjunctival irritation score of 3.3/20.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Published data on the reproductive and developmental toxicity of 6-Amino-*o*-Cresol or 4-Chloro-2-Aminophenol were not found.

Dermal

m-*Aminophenol,* o-*Aminophenol, and* p-*Aminophenol*

The teratogenic potential of hair dyes containing *m*-, *o*-, and/or *p*-aminophenol were determined using rats (Burnett et al. 1976). A dose of 2 ml/kg of oxidative hair dyes containing 0.7% *m*-aminophenol and 1.0% *p*-aminophenol, 0.7% *m*-aminophenol, 0.3% *o*-aminophenol, or 1.0% *N*-methyl-*p*-aminophenol sulfate mixed with an equal volume of 6% hydrogen peroxide or semipermanent hair dyes containing 0.09% and 0.2% *m*-aminophenol and *p*-aminophenol, respectively, or 0.02%, 0.04%, and 0.05% *m*-aminophenol, *p*-aminophenol, and *N*-methyl-*p*-aminophenol sulfate, respectively, were applied topically to the animals on days 1, 4, 7, 10, 13, 16, and 19 of gestation. The hair dyes were not teratogenic or embryotoxic.

Burnett and Goldenthal (1988) conducted a two-generation reproduction study using rats. Twice weekly, 0.5 ml of oxidative hair dye formulations containing 0.7% *m*-aminophenol and 1.0% *p*-aminophenol, 0.7% *m*-aminophenol, 0.3% *o*-aminophenol, or 1.0% *N*-methyl-*p*-aminophenol sulfate mixed with an equal volume of 6% hydrogen peroxide was applied to a shaved area of the back of each animal. Successive applications were made to adjacent areas to minimize dermal irritation. When the rats were 100 days old, they were mated to produce an F_{1a} generation that was eventually used in a carcinogenicity study. The F_0 generation was reduced and re-mated to produce an F_{1b} generation. Rats from the F_{1b} litters were mated after 100 days to produce F_{2a} and F_{2b} litters. Male and female F_2 parents were selected and mated to produce an F_3 generation. However, a viral infection resulted in poor reproductive performance for all groups, including controls, invalidating the results. Dermal irritation consisting of intermittent mild dermatitis was noted during the treatment period in each generation. The topical application of oxidative hair dye formulations did not have an adverse effect on reproductive performance or on the health and survival of the developing fetus and postnatal animals.

Oral

6-Amino-m-*Cresol*

Female Sprague-Dawley rats were dosed orally with 5, 50, or 200 mg/kg 6-Amino-*m*-Cresol from days 6 to 15 of gestation (Hazleton Laboratories 1982). A control (distilled water) and positive control (vitamin A, 15 mg/kg) were also included. The control, positive-control, and 5- and 50-mg/kg groups had

23 animals per group, whereas 26 animals were used in the high-dose group. Rats were killed on day 19 of gestation.

No mortalities were attributed to treatment effects. No clinical changes were observed in any group. Body weight gain of all treated groups was comparable to the control group. No significant changes were observed at necropsy. No effect on pregnancy incidence was observed in the treated groups. The mean number of corpora lutea and the mean number of implantations per dam (preimplantation loss) were comparable to control groups. Postimplantation loss was not affected by 6-Amino-*m*-Cresol and postimplantation loss was lowest in the 200-mg/kg group. The number and sex of the fetuses and the litter and mean fetal weights in the treatment groups were comparable to the control group. Fetal defects, visceral and skeletal variations were the same as the control group. No malformations occurred in the treated groups. The positive control group had marked teratogenic effects: the majority of fetuses had exencephaly. 6-Amino-*m*-Cresol did not elicit embryotoxicity, embryolethality, or teratogenicity.

5-Amino-4-Chloro-o-*Cresol*

Pregnant Wistar/HAN rats (190 to 238 g) were dosed with 5-Amino-4-Chloro-*o*-Cresol hydrochloride in water (10 ml/kg) daily by gavage on days 6 to 15 of pregnancy (period of major organogenesis in the fetus). Four groups of 25 animals each received doses of 0, 20, 100, or 500 mg/kg/day of 5-Amino-4-Chloro-*o*-Cresol hydrochloride. Maternal mortality and body weight gain were recorded. The dams were killed on day 21 of gestation and the fetuses removed for examination. The number of alive and dead fetuses, fetal weight, sex, site of implantation in the uterus, early and late resorptions, and number of corpora lutea were determined. Half of the fetuses were selected at random and examined for visceral and brain abnormalities. The remaining fetuses were examined for abnormalities after staining with alizarin.

The only maternal effect seen was a brown discoloration of the urine. At examination of the fetuses, no developmental toxicity was associated with treatment with 5-Amino-4-Chloro-*o*-Cresol hydrochloride (Henkel KGaA 1994).

5-Amino-6-Chloro-o-*Cresol*

Pregnant Wistar/HAN rats (186 to 234 g) were exposed to 5-Amino-6-Chloro-*o*-Cresol hydrochloride in water daily by gavage on days 6 to 15 of pregnancy (period of major organogenesis in the fetus). Four groups of 25 animals each received doses of 0, 30, 90, or 270 mg/kg/day of 5-Amino-6-Chloro-*o*-Cresol hydrochloride. Maternal mortality and body weight gain were recorded. The dams were killed on day 21 of gestation and the fetuses removed for examination. The number of alive and dead fetuses, fetal weight, sex, site of implantation in the uterus, early and late resorptions, and number of corpora lutea were determined. Half of the fetuses were selected at random and examined for visceral and brain abnormalities. The remaining fetuses were examined for abnormalities after staining with alizarin.

The only maternal effects were slight reduction in feed consumption and reduced body weight gain in the highest dose group. The NOAEL was considered to be 90 mg/kg/day. No developmental toxicity was associated with treatment with 5-Amino-6-Chloro-*o*-Cresol hydrochloride (Henkel KGaA 1994).

4-Amino-m-*Cresol*

Female rats (strain BOR:WISW-SPF TNO) were dosed orally with 10, 40, or 80 mg/kg 4-Amino-*m*-Cresol from days 5 to 15 of gestation (Forschungs GmbH 1984b). A control group was included. Positive proof of sperm in the vaginal smear was considered day 0 of gestation. Each group consisted of 24 animals. Dams were killed on day 20 of gestation.

No abnormal clinical observations were found during the study and no mortalities occurred. Body weight gain and food consumption had no significant intergroup differences. No abnormalities were observed at gross necropsy. No significant differences were observed between groups in mean number of fetuses per dam, left-right intrauterine distribution, sex ratio, birth position, weight, death of fetuses and live birth index, number of resorptions, resorption indices, implantations, postimplantation loss index, corpora lutea and placenta, gravid uteri, and uteri weights. External and skeletal examination of fetuses revealed no malformations. Visceral examination included one fetus in the 40-mg/kg group with hydrocephaly and two fetuses in the 80-mg/kg group with minor visceral anomalies (increased renal pelvic cavitation). The malformation index for all groups was 0, except the 40-mg/kg group, which had a malformation index of 0.56%. The NOAEL was established at the high dose, 80 mg/kg.

4-Amino-2-Hydroxytoluene

Oral administration of $\leq$3% 4-amino-2-hydroxytoluene produced maternal toxicity but was not teratogenic (Elder 1989).

m-*Aminophenol and* p-*Aminophenol*

Oral administration of 250 mg/kg *p*-aminophenol resulted in reduced maternal body weight gains and teratogenicity in offspring (external, skeletal, and visceral malformations) in a study using rats (Elder 1988). Chronic feeding of 0.7% *p*-aminophenol in the diet of rats produced embryotoxicity mediated by maternal toxicity. Chronic feeding of $\leq$1% *m*-aminophenol to rats resulted in maternal toxicity during gestation, but teratogenic effects were not observed. Oral administration of 100 to 200 mg/kg *p*-aminophenol to gravid hamsters did not produce teratogenic effects.

Parenteral

m-*Aminophenol,* o-*Aminophenol, and* p-*Aminophenol*

Elder (1988) reported that intravenous and i.p. administration of 100 to 200 mg/kg *p*-aminophenol induced fetal malformations; i.p. administration of *o*-aminophenol to hamsters resulted in teratogenic effects; but that no conclusive evidence was found for *m*-aminophenol using i.p. administration.

GENOTOXICITY

In Vitro

*6-Amino-*m*-Cresol*

The mutagenic potential of 6-Amino-*m*-Cresol was evaluated in an Ames test using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 (Noser 1979a). Concentrations of 30 to 1000 μg 6-Amino-*m*-Cresol, alone and with equal amounts of 6% hydrogen peroxide, were tested with and without metabolic activation. Negative and positive controls were used. 6-Amino-*m*-Cresol was slightly mutagenic towards *S. typhimurium* TA100 with and without metabolic activation. It was not mutagenic towards the other strains.

Saccharomyces cerevisiae diploid D7 cell cultures were exposed to 0.1 ml of 6-Amino-*m*-Cresol in DMSO at concentrations of 0.6, 3.0, and 15.0 μg/ml with and without metabolic activation (Bootman 1984a). Negative (DMSO) and positive (ethyl methanesulphonate) controls were used. 6-Amino-*m*-Cresol was highly toxic to the yeast cells, but it did not induce increases in the frequency of convertant or aberrant colonies with or without metabolic activation.

Mouse lymphoma L5178Y cells were treated for 2 h with 400 ml of 12.5 to 200 μg/ml 6-Amino-*m*-Cresol in DMSO with and without metabolic activation (Martin 1983). DMSO was used as the negative control and benzopyrene with metabolic activation and 4-nitroquinoline-1-oxide without metabolic activation were used as the positive controls. All microtitre plates were incubated for 2 weeks, after which wells with viable clones were counted. Cell viability was measured by adding ouabain and 6-thioguanine to cell suspensions 48 h and 7 days after treatment, respectively. 6-Amino-*m*-Cresol did induce an increase in mutation to both ouabain and 6-thioguanine resistance in the presence of metabolic activation; however, the increase was not considered significant with or without metabolic activation.

The clastogenic potential of 6-Amino-*m*-Cresol hemisulfate was determined using cultured male human peripheral lymphocytes (Bootman 1984b). Cell cultures were incubated for 24 h with 25 μl of the test compound dissolved in DMSO at concentrations of 0.6, 3.0, and 15.0 μg/ml with and without metabolic activation. DMSO was used as the negative control and cyclophosphamide with metabolic activation was used as the positive control. 6-Amino-*m*-Cresol hemisulfate did not significantly increase the number of aberrations as compared to controls.

*4-Amino-*m*-Cresol*

The mutagenic potential of 4-Amino-*m*-Cresol was evaluated in an Ames test using *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 (Noser 1979b). Concentrations of 15 to 600 μg/plate 4-Amino-*m*-Cresol, alone and with equal amounts of 6% hydrogen peroxide, were tested with and without metabolic activation. Negative and positive controls were used. 4-Amino-*m*-Cresol was not mutagenic with or without metabolic activation.

In an unscheduled DNA synthesis (UDS) assay, male rat primary hepatocytes were incubated with 1.0, 3.33, 10.0, 33.33, or 100.0 μg/ml 4-Amino-*m*-Cresol in DMSO (Miltenburger 1986). Negative controls were untreated or incubated with solvent and positive controls were incubated with 7,12-dimethylbenz(a) anthracene. 4-Amino-*m*-Cresol did not induce UDS in rat hepatocytes.

4-Chloro-2-Aminophenol

The mutagenic potential of 4-Chloro-2-Aminophenol in DMSO was determined in a preincubation assay (Zeiger et al. 1988). Concentrations of 10 to 1500 μg/plate were tested using *S. typhimurium* strains TA100, TA1535, TA97, and TA98 without and with metabolic activation. 4-Chloro-2-Aminophenol was weakly mutagenic.

*5-Amino-4-Chloro-*o*-Cresol*

The mutagenic potential of 5-Amino-4-Chloro-*o*-Cresol hydrochloride was evaluated in an Ames test using *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 (Henkel KGaA 1994). Concentrations of 4 to 2500 μg/plate with the 5-Amino-4-Chloro-*o*-Cresol hydrochloride dissolved in water and 75 to 1200 μg/plate with the 5-Amino-4-Chloro-*o*-Cresol (the free base) dissolved in DMSO were tested with and without metabolic activation by Aroclor 1254–induced rat liver enzymes. Positive controls were used as follows: Sodium azide for TA 100 and TA 1535; 9-aminoacridine for TA 1537; 4-nitro-*o*-phenylenediamine for TA 98 and TA 1538; and 2-aminoanthracene for all strains. Toxic effects were noted at the greatest concentration tested (2500 μg/plate). Table 3 has a summary of the results of this study. On the basis of these data, the investigators concluded that the free base was mutagenic with metabolic activation.

V79 Chinese hamster lung cells were used to examine the mutagenicity of 5-Amino-4-Chloro-*o*-Cresol hydrochloride. Mutations to 6-thioguanine resistance at the *HGRPT* locus with

TABLE 3
5-Amino-4-Chloro-*o*-Cresol Ames test results (Henkel KGaA 1994)

	With metabolic activation		Without metabolic activation	
Strain	Hydrochloride in water	Free base in DMSO	Hydrochloride in water	Free base in DMSO
TA 98	Neg	Weak pos	Neg	Neg
TA 100	Weak pos	Pos	Neg	Neg
TA 1535	Neg	Neg	Neg	Neg
TA 1537	Neg	Weak pos	Neg	Neg
TA 1538	Neg	Pos	Neg	Neg

Neg, negative; Pos, positive.

TABLE 4
5-Amino-6-Chloro-*o*-Cresol Ames test results (Henkel KGaA 1996)

Strain	With metabolic activation		Without metabolic activation
	Phenobarbital	Aroclor 1254	
TA 98	Neg	Pos	Neg
TA 100	Neg	Pos	Neg
TA 1535	Neg	Neg	Neg
TA 1537	Neg	Neg	Neg
TA 1538	Neg	Pos	Neg

Neg, negative; Pos, Positive.

and without metabolic activation were measured. 5-Amino-4-Chloro-*o*-Cresol hydrochloride dissolved in ethanol at 6 to 60 μg/ml without metabolic activation and 55 to 550 μg/ml with metabolic activation (Aroclor 1254–induced rat liver enzyme fraction) were used. Ethyl methanesulfonate (EMS) and dimethylbenz[a]anthracene (DMBA) served as positive controls. At no concentration or metabolic activation status were any increases seen in the number of mutations (Henkel KGaA 1994).

5-Amino-6-Chloro-o-*Cresol*

The mutagenic potential of 5-Amino-6-Chloro-*o*-Cresol hydrochloride was evaluated in an Ames test using *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 (Henkel KGaA 1996). Concentrations of 4 to 2500 μ g/plate with the 5-Amino-6-Chloro-*o*-Cresol hydrochloride was tested with and without metabolic activation by Aroclor 1254 or phenobarbital induced rat liver enzymes. Positive controls were used as follows: Sodium azide for TA 100 and TA 1535; 9-aminoacridine for the other strains. Table 4 presents the results of this study. On the basis of these data, the investigators concluded that 5-Amino-6-Chloro-*o*-Cresol hydrochloride was mutagenic with metabolic activation.

V79 Chinese hamster lung cells were used to examine the mutagenicity of 5-Amino-6-Chloro-*o*-Cresol hydrochloride. Mutations to 6-thioguanine resistance at the *HGRPT* locus with and without metabolic activation were measured. 5-Amino-4-Chloro-*o*-Cresol hydrochloride dissolved in ethanol at 0, 35, 100, 200, and 300 μg/ml without metabolic activation and 0, 25, 100, 200, and 300 μg/ml with metabolic activation (Aroclor 1254–induced rat liver enzyme fraction) were used. EMS and DMBA served as positive controls. At concentrations $\geq$50 μg/ml, the plating efficiency of the cells was slightly reduced. At no concentration or metabolic activation status were any increases seen in the number of mutations (Henkel KGaA 1996).

V79 Chinese hamster lung cells were used to examine the mutagenicity of 5-Amino-6-Chloro-*o*-Cresol hydrochloride at concentrations from 10 to 1100 μg/ml. Chromosomes were prepared 7 (high dose), 18 (low, medium, and high dose), and 28 (high dose) h after the start of a 4-h treatment. Treatment was done with and without Aroclor 1254–induced rat liver enzymes. EMS was used as a positive control. Concentrations of 1000 and 3000 μg/ml were toxic in range finding studies, with and without metabolic activation. Although no chromosome aberrations were seen at 7 h, chromosome aberrations were increased in all dose groups at 18 h and at 28 h. The authors concluded that 5-Amino-6-Chloro-*o*-Cresol hydrochloride does induce chromosome aberrations in the V79 line independent of metabolic activation (Henkel KGaA 1996).

Unscheduled DNA synthesis (a measure of DNA damage) was measured in rat liver hepatocytes exposed to 5-Amino-6-Chloro-*o*-Cresol hydrochloride at concentrations ranging from 6.67 to 2000 μg/ml. Six cultures were used for each concentration and the experiments were repeated three times. Cells were incubated without the test compound for 1 h, at which time tritiated thymidine and the test substance were added and incubated a further 3 h. 2-Acetylaminofluorene (2-AAF) served as a positive control. Cells were washed, nuclei isolated, and the incorporated radioactivity was measured. Total DNA content was determined colorimetrically. No indications of a dose-related increase in unscheduled DNA synthesis were observed (Henkel KGaA 1996).

In Vivo

6-Amino-m-*Cresol*

In a micronucleus test, male CD-1 mice (10 per group) were dosed orally with 30, 150, or 750 mg/kg 6-Amino-*m*-Cresol in 0.5% carboxymethylcellulose at a volume of 10 ml/kg once daily for 2 days (Holmstroem 1980). The mice were dosed during two separate studies 6 and 30 h before they were killed. The vehicle was used as a negative control and 100 mg/kg cyclophosphamide was used as a positive control. Body weights did not vary by more than 1 g during the study. 6-Amino-*m*-Cresol did not increase the frequency of micronuclei.

In another micronucleus test, groups of six male and female NMRI mice were orally dosed with 500 mg/kg 6-Amino-*m*-Cresol in polyethylene glycol 400 (Völkner and Heidemann 1991). Three negative and one positive control (cyclophosphamide) were dosed orally once at 10 ml/kg. Bone marrow smears for the treated groups and negative control were prepared 24, 48, and 72 h post treatment. Bone marrow smears for the positive control were prepared 24 h post treatment. 6-Amino-*m*-Cresol did not induce micronuclei.

Groups of five male and five female NMRI mice were dosed orally with 666 mg/kg 6-Amino-*m*-Cresol in carboxymethylcellulose in a third micronucleus test (Leimbeck and Grötsch 1991). One negative and one positive control (cyclophosphamide, 40 mg/kg) were used. Bone marrow smears were evaluated 24, 48, and 72 h post administration. Again, 6-Amino-*m*-Cresol did not induce micronuclei in bone marrow cells.

A chromosome aberration study was conducted using groups of five male and five female Chinese hamsters (King and

Harnasch 1991). The animals were dosed once orally with 3200 mg/kg 6-Amino-*m*-Cresol in 4% gum arabic, and slides were prepared 6, 24, and 48 h post treatment. One negative control group was dosed with 20 ml of 4% gum arabic per kg body weight and one positive control was dosed i.p. with 30 mg/kg cyclophosphamide. Preparations from the positive control group were made at 24 h. A cytotoxic effect was observed, which indicated a strongly decreased ratio of polychromatic and normochromatic erythrocytes in the bone marrow (55% reduction compared to control animals). 6-Amino-*m*-Cresol did not induce chromosome aberrations in Chinese hamster bone marrow cells.

A bromodeoxyuridine pellet was implanted subcutaneously into male CD rats, and 2 h later groups of five animals were given a single oral dose of 60, 192, or 600 mg/kg 6-Amino-*m*-Cresol hemisulfate in distilled water (McGregor 1985). A negative-control group was given vehicle and a positive-control group was dosed with 5 mg cyclophosphamide. The animals were injected with colchicine 20 h after implantation, and killed 2 h after injection. 6-Amino-*m*-Cresol hemisulfate did not cause sister chromatid exchanges (SCEs) in rat bone marrow chromosomes.

An unscheduled DNA synthesis assay was performed using groups of five male Wistar Hanlbm:WIST (SPF) rats (Fautz 1994). The animals were given a single oral dose of 6-Amino-*m*-Cresol in 0.5% aqueous carboxymethylcellulose at a volume of 10 ml/kg. For the 2 h treatment, a dose of 1500 mg/kg was given and for the 16 h treatment, doses of 150 and 1500 mg/kg were used. A negative control (carboxymethyl cellulose) and a positive control, 100 mg/kg 2-AAF, were used. One of the animals in the 1500-mg/kg dose group died within 16 h of treatment and the other animals in the group had signs of toxicity. Additionally, the hepatocyte viability of two animals out of the 1500-mg/kg group was decreased. 6-Amino-*m*-Cresol did not induce UDS.

4-Amino-m-*Cresol*

In another micronucleus test, groups of six male and six female NMRI mice were given a single oral dose of 100, 333, or 1000 mg/kg 4-Amino-*m*-Cresol in DMSO (Miltenburger and Völkner 1988). Vehicle was used as the negative control and cyclophosphamide was used as the positive control. Femoral bone marrow cells were prepared 24 h after dosing for all groups and 48 and 72 h after dosing for the high-dose and control groups. 4-Amino-*m*-Cresol did not induce micronuclei.

In a micronucleus test, CD-1 mice were dosed with 20, 100, or 500 mg/kg 4-Amino-*m*-Cresol (Holmstroem 1980). The mice were dosed during two separate studies 6 and 30 h before they were killed. The vehicle control was 0.5% carboxymethylcellulose. The positive control was cyclophosphamide, which induced a small but significant increase in micronucleus frequency. Body weights did not vary by more than 1 g during the study. 4-Amino-*m*-Cresol did not increase the frequency of micronuclei in polychromatic erythroblasts.

In an SCE assay, groups of $\leq$25 male Chinese hamsters were dosed orally with 100, 300, 1000, 1500, or 2000 mg/kg or i.p. with 10, 30, 100, 300, or 400 mg/kg 4-Amino-*m*-Cresol hemisulfate in double distilled water (Bracher et al. 1984). Water was used as a negative control and 2-AAF was used as a positive control. Doses of 1500 and 2000 mg/kg p.o. and 400 mg/kg i.p. had cytotoxic effects, and a dose of 500 mg/kg i.p. was "partly lethal." 4-Amino-*m*-Cresol hemisulfate did not cause SCEs, regardless of administration.

A UDS assay was performed in which groups of five male Wistar rats were dosed with 4-Amino-*m*-Cresol in "aqua bidest" at a dose of 1000 mg/kg for the 4 h treatment and doses of 60 and 600 mg/kg for the 16 h treatment (Fautz and Völkner 1991). A negative control and a positive control (substances not specified) was used. 4-Amino-*m*-Cresol did not induce UDS.

Five male Wistar rats per group were dosed with 1000 mg/kg 4-Amino-*m*-Cresol and killed 4 hours post treatment and 60 and 600 mg/kg and killed 16 hours posttreatment (Fautz and Völkner 1991b). The negative-control group received DMSO/PEG 400 and the positive-control group received 2-AAF. The rats were killed at the designated times by liver perfusion. Three animals from each group were used in the UDS assay. Hepatocytes were cultured with ^{3}H-radiolabeled thymidine (^{3}HtdR) for 4 h. The hepatocytes were washed and incubated overnight prior to autoradiography. The nuclear and net grain counts of the treated groups were in the range of the corresponding controls, therefore a statistical evaluation was not performed. 4-Amino-*m*-Cresol did not induce DNA damage leading to repair synthesis in the hepatocytes of treated rats.

5-Amino-4-Chloro-o-*Cresol*

An in vivo micronucleus test for chromosome mutations was conducted using adult CFW1 mice (20–32 g). Seven male and seven female mice were used at each dose. The test substance was dissolved in water at doses of 50, 250, and 500 mg/kg of 5-Amino-4-Chloro-*o*-Cresol hydrochloride was administered once by gavage. Bone marrow extracted from the femurs was prepared 24, 48, and 72 h after dosing in the case of the highest dose group and at 24 h for the other two dose groups. Endoxan® was the positive control and the vehicle was the negative control. Analysis was done of 1000 polychromic erythrocytes per animal. No induced micronuclei were found at any dose. The investigators concluded that 5-Amino-4-Chloro-*o*-Cresol hydrochloride was not mutagenic in this assay (Henkel KGaA 1994).

5-Amino-6-Chloro-o-*Cresol Hydrochloride*

An in vivo micronucleus test for chromosome mutations was conducted using adult OF1 mice (28.7–37.8 g for males and 21.6–30.0 g for females). Five male and five female mice were used. The test substance was dissolved in water and administered once by gavage to a final dose of 1200 mg/kg of 5-Amino-6-Chloro-*o*-Cresol hydrochloride. Bone marrow extracted from the femurs was prepared 24, 48, and 72 h after dosing in the case of the highest dose group and at 24 h for the other two dose groups. Cyclophosphamide (10 mg/kg) was the positive control and the vehicle was the negative control. Analysis was done of

1000 polychromic erythrocytes per animal. The ratio of chromatic/polychromatic erythrocytes was slightly increased, suggesting some toxicity to the bone marrow, but the investigators concluded that 5-Amino-6-Chloro-*o*-Cresol hydrochloride was not mutagenic in this assay (Henkel KGaA 1994).

4-Amino-2-Hydroxytoluene

In Ames tests, 4-amino-2-hydroxytoluene was not mutagenic using *S. typhimurium* strain TA1535 without and with metabolic activation; 4-amino-2-hydroxytoluene was not mutagenic in some studies using strains TA98 and TA100 without and with metabolic activation, but was mutagenic in one study towards strains TA98, TA97, and TA100 (Elder 1989). Negative results were obtained in a micronucleus assay and a dominant lethal study using 4-amino-2-hydroxytoluene. No significant effect on SCEs or increase in chromosomal aberrations was observed in human lymphocytes obtained from subjects that repeatedly dyed their hair with a formulation containing 4-amino-2-hydroxytoluene.

p-*Aminophenol*

Elder (1988) reported that *p*-Aminophenol was strongly mutagenic in an assay for SCEs (human peripheral blood lymphocytes, $\leq 10^{-4}$ M), was mutagenic in a DNA synthesis inhibition assay (Epstein-Barr virus–transformed lymphoblastoid cells, j0.5 mM), three assays for DNA structural alterations (human lymphoblastoid cells, 0.05 to 0.5 mM; mouse bone marrow cells; plant cells), two erythrocyte micronucleus tests ($\leq$2 mmol/kg; 3%), and a sperm head abnormality test (200 to 400 mg/kg), was slightly mutagenic in an Ames assay without metabolic activation and one assay for SCEs, and was nonmutagenic in an Ames assay without and with metabolic activation ($\leq$2 μmol/plate), an *Escherichia coli* genetic repair assay, two assays for SCEs (Chinese hamster bone marrow cells, 5 mg/kg; metaphase human fibroblasts, 5 to 50 μM), one erythrocyte micronucleus test (0.5%), a thymidine kinase reversion assay (1% with metabolic activation), and a sperm head abnormality test (0.5 to 2.0 mmol/kg).

m-*Aminophenol*

Elder (1988) also reported that *m*-Aminophenol was mutagenic in an assay for DNA structural alterations (human lymphocytes); was slightly mutagenic in an assay for SCEs (human lymphocytes, 6.6 μg/ml); and was nonmutagenic in an Ames assay ($\leq$1 mg/ml agar with metabolic activation), an *E. coli* genetic repair assay, a DNA synthesis inhibition assay (rat hepatocytes, $\leq$500 nmol/ml), an assay for DNA structural alterations (human lymphocytes, 6.6 μg/ml), two SCE induction assays (Chinese hamster cells, 0.5–2 $\times$ 10^{-2} mM; Chinese hamster bone marrow cells, 5 mg/kg), two erythrocyte micronucleus tests (0.5–2 mmol/kg; 0.5%), a dominant lethal assay ($\leq$1%), and a sperm head abnormality test (0.5 to 2 mmol/kg). Also, no significant effect on SCEs or increase in chromosomal aberrations was observed in human lymphocytes obtained from subjects that repeatedly dyed their hair with a formulation containing *p*- or *m*-aminophenol (Elder 1988)

o-*Aminophenol*

Elder (1988) reported that *o*-Aminophenol was mutagenic in one Ames assay (7 to 100 μg/ml with metabolic activation), an *E. coli* genetic repair assay, three assays for SCE induction (human fibroblasts, 0.01 to 0.3 mM; Chinese hamster cells, 0.5–2 $\times$ 10^{-2} mM; human lymphocytes, 1.6 to 6.6 μg/ml), an erythrocyte micronucleus test (0.5 to 2 mmol/kg), and a sperm head abnormality test (0.5 to 2 mmol/kg) and was nonmutagenic in two Ames assays (0.5 to 2.0 μg/plate without and with metabolic activation; with metabolic activation), a DNA synthesis inhibition assay (rat hepatocytes, $\leq$100 nmol/ml), one SCE induction assay (Chinese hamsters, 5 mg/kg), and an assay for DNA structural alterations (implanted Ehrlich ascites tumor cells).

CARCINOGENICITY

Published data on the carcinogenicity of the ingredients reviewed in this safety assessment were not found. Data from previous safety assessments of related ingredients are summarized.

m-*Aminophenol,* o-*Aminophenol, and* p-*Aminophenol*

The carcinogenic potential of an oxidative hair dye containing 0.5% and 1.5% *p*-amino-*o*-cresol and *p*-aminophenol, respectively, was determined using mice (Jacobs et al. 1984). A dose of 0.5 ml of the dye mixed with an equal volume of 6% hydrogen peroxide was applied to the skin of each mouse once weekly for 20 months. The oxidative dye was not carcinogenic.

The carcinogenic potential of hair dyes containing *m*-, *o*-, and/or *p*-aminophenol were determined using mice (Burnett et al. 1980). A dose of 0.05 ml of oxidative hair dyes containing 0.7% *m*-aminophenol and 1.0% *p*-aminophenol, 0.7% *m*-aminophenol, 0.3% *o*-aminophenol, or 1.0% *N*-methyl-*p*-aminophenol sulfate mixed with an equal volume of 6% hydrogen peroxide were applied once weekly for 21 months and 0.05 ml of semipermanent hair dyes containing 0.09% and 0.2% *m*-aminophenol and *p*-aminophenol, respectively, or 0.02%, 0.04%, and 0.05% *m*-aminophenol, *p*-aminophenol, and *N*-methyl-*p*-aminophenol sulfate, respectively, were applied once weekly for 23 month. The hair dyes were not carcinogenic, and toxicity was not observed.

Burnett and Goldenthal (1988) also conducted a study to determine the carcinogenic potential of oxidative hair dye formulations containing 0.7% *m*-aminophenol and 1.0% *p*-aminophenol, 0.7% *m*-aminophenol, 0.3% *o*-aminophenol, or 1.0% *N*-methyl-*p*-aminophenol sulfate using the F_{1a} generation of rats from their reproduction study that was previously summarized. The formulations were mixed with equal volumes of 6% hydrogen peroxide and twice weekly a dose of 0.5 ml was applied topically to a shaved area of the back for approximately 2 years. Successive applications were made to adjacent areas to minimize dermal irritation.

The incidence of mammary gland adenomas was significantly increased for the female test animals as compared to the animals in one of three control groups; however, this value was not considered statistically different from the other two control groups. The incidence of pituitary adenomas significantly increased for female test animals as compared to all three control groups. The researchers noted that the "incidence of this tumor is known to be high and variable in untreated female Sprague-Dawley rats. The fact that no pituitary carcinomas occurred in this group suggests that the distribution of these tumors was not related to the experimental treatments." The oxidative hair dye formulations were not considered carcinogenic.

CLINICAL ASSESSMENT OF SAFETY

Irritation and Sensitization

Published data on the clinical irritation and sensitization potential of 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, or 5-Amino-6-Chloro-*o*-Cresol were not found.

4-Chloro-2-Aminophenol

Thirty-one factory workers were patch tested with 4-Chloro-2-Aminophenol, as well as with four other compounds (*p*-aminophenol, p-nitrophenol, *p*-dichloronitrobenzene, and 3′-chlorodiphenylamine-2-carboxylic acid) used or produced at the factory (Naniwa 1979). (4-Chloro-2-Aminophenol, *p*-amin ophenol, and 3′-chlorodiphenylamine-2-carboxylic acid are amino derivatives of aromatic compounds and *p*-nitrophenol and *p*-dichloronitrobenzene are nitro derivatives of them.) Using adhesive plasters, 0.1%, 0.5%, and 1.0% 4-Chloro-2-Aminophenol (and the other four compounds) in petrolatum was applied to the back of each subject for 48 h. The tests sites were scored 20 min after removal of the patches. A challenge test was performed by dropping 0.1 ml of 0.1% dinitrochlorobenzene (DNCB) in acetone onto the flexural antibrachium of each person, and the reaction was evaluated 48 h after application. A group of five control subjects was tested in the same manner.

Of the 31 subjects tested, 7 had positive reactions to 0.1%, 0.5%, and 1.0% 4-Chloro-2-Aminophenol, 6 had positive reactions to 0.5% and 1.0%, 2 had positive reactions to 0.1% and 0.5%, 1 had a positive reaction to 1.0% only, and one had a positive reaction to 0.1% and 1.0%. Six of the seven subjects that reacted to all three concentrations of 4-Chloro-2-Aminophenol had been directly exposed to it on repeated occasions. Some cross-sensitization might have occurred between 4-Chloro-2-Aminophenol and *p*-aminophenol (four cases), *p*-nitrophenol (one case), *p*-dichloronitrobenzene (three cases), and 3′-chlorodiphenylamine-2-carboxylic acid (two cases). None of the test subjects had a cross-sensitization reaction with DNCB. None of the control subjects had a primary irritation reaction to any of the tested compounds.

4-Amino-2-Hydroxytoluene

In modified Draize repeat-insult patch tests (RIPTs), two aqueous solutions containing 2.0% 4-amino-2-hydroxytoluene produced one (although not reconfirmed at challenge) and two significant cases of dermatitis using 23 and 31 subjects, respectively (Elder 1989). In two semiocclusive (open) RIPTs with 3% *m*-aminophenol, slight irritation during induction and no sensitization reactions at challenge were observed in one study and some irritation and a low degree of sensitization in 2/99 subjects was observed in the other study.

EPIDEMIOLOGY

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

Hair dyes may be broadly grouped into oxidative (permanent) and direct (semipermanent) hair dyes. The oxidative dyes consist of precursors mixed with developers to produce color, although direct hair dyes are a preformed color. The ingredients addressed in this safety assessment are oxidative hair dyes.

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

In 2003, an updated review of the available epidemiology literature was prepared (Helzlsouer, Rollison, and Pinney 2003). This review considered 83 literature citations available since the IARC review. The authors found that hair dye exposure assessment ranged from ever/never use to information on type, color, duration and frequency of use.

The authors found insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. The review highlighted well-designed studies with an exposure assessment that included hair dye type, color, and frequency or duration of use, which found associations between personal hair dye use and development of bladder cancer, non-Hodgkin's lymphoma, and multiple myeloma. These findings, however, were not consistently observed across studies. The authors concluded that the available evidence is insufficient to conclude a causal association between personal hair dye use and bladder cancer, non-Hodgkin's lymphoma, and multiple myeloma. With respect to other cancers, including leukemia, breast cancer, or childhood cancers, and autoimmune disease or adverse developmental/reproductive effects, the

authors concluded that the evidence also did not demonstrate a causal association with hair dye use.

A case-control study (Gago-Dominguez et al. 2001, 2003), described in this 2003 review, did suggest a possible genetically susceptible subgroup, which detoxify arylamines to a lower degree than the general population. The study authors hypothesized that this subgroup may be at greater risk of bladder cancer from hair dye exposure. The review authors noted that these results were based on small sample sizes.

The 2003 review authors recommended the replication of studies to better understand the observed associations, but concluded that the available evidence is insufficient to conclude the association between personal hair dye use and the health outcomes discussed is causal.

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

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

The Panel recognizes that hair dye epidemiological studies do not address the safety of individual hair dyes, but is concerned that studies have demonstrated an association between use of oxidative/permanent hair dyes and some cancer endpoints. The Panel, therefore, strongly supports the need to replicate these studies, along with further studies to examine the possibility of susceptible subpopulations. Additional studies examining bladder cancer, non-Hodgkin's lymphoma, and multiple myeloma and hair dye use are underway and it is the intent of the CIR Expert Panel to periodically review hair dye epidemiological studies and update this section.

Occupational

4-Chloro-2-Aminophenol

Blood samples were taken from 21 workers that handled 4-Chloro-2-Aminophenol (and other compounds) (Tomoda, Tomioka, and Minami 1989). Half-oxidized hemoglobins, such as $(\alpha^{2+}\beta^{3+})_2$ and $(\alpha^{3+}\beta^{2+})_2$, and methemoglobin were significantly increased in circulating erythrocytes of some workers.

Exposure Assessment

*5-Amino-4-Chloro-*o*-Cresol*

Considering that 5-Amino-4-Chloro-*o*-Cresol hydrochloride is used in oxidative hair dye formulations up to a maximum concentration of 2%, Henkel KGaA (1994) assessed the risks that such exposure might pose. Dilution with an oxidant 1:1 reduces the available concentration to 1%. It was estimated that a maximum of 100 ml of this dyeing mixture would be applied monthly. It was further noted that color development is completed within 30 min and the resulting oxidized hair dye is fixed at the hair cortex, with any excess rinsed off (80% to 90% of the dyeing mixture).

From the available percutaneous absorption data in rats (Henkel KGaA 1994), in which dilution with an oxidizer was done to produce a 1.85% hair dye solution and rinsing off after 30 min exposure was done, an intake of 5-Amino-4-Chloro-*o*-Cresol hydrochloride of 5.21 μg/cm^2 was determined. Assuming a scalp surface of 500 cm^2, the total absorbed hair dye would be 2.6 mg. This quantity may be extrapolated to 2.8 mg if a hair dye solution at 2% were applied. Using this latter value and considering a 60-kg user, the dose is 47 μg/kg. Comparing this dose with, for example, the 180-mg/kg dose reported to produce no observable effects in a 90-day oral toxicity study in rats, these investigators concluded a substantial safety factor was available for 5-Amino-4-Chloro-*o*-Cresol.

*5-Amino-6-Chloro-*o*-Cresol*

Considering that 5-Amino-6-Chloro-*o*-Cresol hydrochloride is used in oxidative hair dye formulations up to a maximum concentration of 2%, Henkel KGaA (1996) assessed the risks that such exposure might pose. Dilution with an oxidant 1:1 reduces the available concentration to 1%. It was estimated that a maximum of 100 ml of this dyeing mixture would be applied monthly. It was further noted that color development is completed within 30 min and the resulting oxidized hair dye is fixed at the hair cortex, with any excess rinsed off (80 to 90% of the dyeing mixture).

From the available percutaneous absorption data in rats (Henkel KGaA 1996) in which dilution with an oxidizer was done to produce a 1.14% hair dye solution and rinsing off after 30 min exposure was done, only 0.116% of 5-Amino-6-Chloro-*o*-Cresol hydrochloride was absorbed. Assuming a scalp surface of 500 cm^2, 100 ml of hair dye mixture applied, concentration of dye of 1.14%, and absorption of 0.116%, the total absorbed hair dye can be calculated to be only 8.87 μg. This quantity may be extrapolated to 17.75 μg if a hair dye solution at 2% were applied. Using this latter value and considering a 60-kg user, the dose is 0.3 μg/kg. Comparing this dose with, for example, the 50-mg/kg dose that was reported to produce no observable effects in a 90-day oral toxicity study in rats, the investigators concluded that a substantial safety factor was available for 5-Amino-6-Chloro-*o*-Cresol.

SUMMARY

6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Chloro-2-Aminophenol function as hair colorants. 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol are identified as oxidative hair dyes, that is, they are combined with an oxidizing agent before being applied to the hair. Information is not available to determine if 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, and 5-Amino-4-Chloro-*o*-Cresol

are used only in oxidative hair dyes or have application as nonoxidative (commonly referred to as semipermanent) hair dyes.

In 1998, frequency of use data submitted by FDA indicated that 6-Amino-*m*-Cresol was used in two hair dye formulations. More recent data available from the industry indicate that 6-Amino-*m*-Cresol was used at 2.4%, 6-Amino-*o*-Cresol was used at 0.7%, and 4-Amino-*m*-Cresol was used at 0.3% in 1999. Recent data from industry also reports that 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol were used at a maximum concentration of 2% in oxidizing hair dyes, which is effectively reduced to 1% with the addition of oxidizing agents.

5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol do not absorb significant UV radiation in the UVB region and none in the UVA region, although 4-Amino-*m*-Cresol had a symmetrical UV absorption peak at 300 nm. Both 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol produce virtually a single peak in HPLC and no small peaks were identified as *m*-cresol. 4-Amino-*m*-Cresol did not contain *m*-cresol when analyzed using HPLC.

Percutaneous penetration of 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol alone was significant, but when combined with oxidative developer, the absorption was extremely low. Both of these dyes are excreted rapidly via the urine.

The hair dyes containing 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Chloro-2-Aminophenol, 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* of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The following caution statement should be displayed conspicuously on the labels of coal tar hair dyes:

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

Repeated exposure of animal skin to 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol failed to produce any cumulative irritation and single exposures up to 10% were not irritating to animal skin.

The response of leukocytes from guinea pigs using the LAI technique suggested that cross-sensitization might occur between 4-Chloro-2-Aminophenol and *p*-aminophenol. However, in testing using guinea pigs in which induction was with 4-Chloro-2-Aminophenol and the animals were challenged first with 4-Chloro-2-Aminophenol and then *p*-aminophenol, animals reacted to 4-Chloro-2-Aminophenol but not *p*-amino phenol. In clinical testing using factory workers, some cross-sensiti zation was observed between 4-Chloro-2-Aminophenol and *p*-aminophenol, as well as *p*-nitrophenol, *p*-dichloronitrobenzene, and 3′-chlorodiphenylamine-2-carboxylic acid. Guinea pig maximization tests of 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol combined with oxidizer demonstrate no sensitization.

Ocular exposure of animals to undiluted 5-Amino-4-Chloro-*o*-Cresol was irritating, but exposure to a 5% solution produced no irritation. Only minor irritation was observed with 5% 5-Amino-6-Chloro-*o*-Cresol.

Subchronic toxicity testing in animals using 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Amino-*m*-Cresol did not yield any adverse reactions.

6-Amino-*m*-Cresol and 4-Amino-*m*-Cresol were generally negative in in vitro and in vivo mutagenicity tests. The only exception was 6-Amino-*m*-Cresol was slightly mutagenic in an Ames assay towards *S. typhimurium* strain TA100 with and without metabolic activation. 4-Chloro-2-Aminophenol was weakly mutagenic in a preincubation assay. 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol were positive in some Ames test strains, but were negative in the HGPRT test in mammalian cells. 5-Amino-4-Chloro-*o*-Cresol did not induce chromosome aberrations in mammalian cells, but 5-Amino-6-Chloro-*o*-Cresol induced chromosome aberrations in mammalian lung cells but not in bone marrow erythrocytes. Neither of these hair dyes induced unscheduled DNA synthesis.

5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, 6-Amino-*m*-Cresol and 4-Amino-*m*-Cresol were not developmental toxins.

An exposure assessment that compared likely exposure levels of 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol with adverse effects data found that exposure would be several orders of magnitude below NOAEL levels.

DISCUSSION

The Expert Panel recognizes that irritation and sensitization data on 6-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, 4-Amino-*m*-Cresol, and 4-Chloro-2-Aminophenol are absent from this report. However, the hair dyes containing the ingredients included in this report, 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* of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Expert Panel expects that following this procedure will identify individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures.

The information available on the use of 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol in hair dye formulations indicate that these ingredients are reacted with a developer and are not available for absorption into the skin of the scalp. These compounds, when tested alone, are moderate skin sensitizers, but when combined with the developer, these ingredients are not sensitizers in animal tests. In addition, no toxicologically significant impurities are present with these two ingredients. This information, coupled with the available animal test data,

support the safety of 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol for use in oxidative hair dyes.

Were 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol to have application in nonoxidative (semipermanent) hair dyes, there is concern about the potential for skin sensitization because these ingredients are moderate sensitizers. Because individuals would be pretested to determine if they would develop skin sensitization and because there is an absence of any significant systemic toxic effects in animal tests, the Panel believes that these two ingredients could be used safely in semipermanent hair dyes. Even though there is currently no use of these ingredients as semipermanent hair dyes, the Panel believes it useful to conclude that they could be used safely.

Although 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol appear to be used only in oxidative hair dyes, it is not clear whether 6-Amino-*m*-Cresol, 4-Amino-*m*-Cresol, 6-Amino-*o*-Cresol, and 4-Chloro-2-Aminophenol are used solely in oxidative hair dyes where they would be reacted with a developer and would not be available for absorption into the skin. Therefore, the Expert Panel has considered each ingredient separately for use in oxidative hair dyes and in semi-permanent hair dyes.

Because 6-Amino-*m*-Cresol, 4-Amino-*m*-Cresol, 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol would be chemically reacted with a developer in oxidative hair dyes, and because the available information has consistently shown that such reactions make the starting ingredient unavailable for skin absorption, the CIR Expert Panel believes these ingredients would present no safety concerns if used in oxidative hair dyes.

The use of 6-Amino-*m*-Cresol, 4-Amino-*m*-Cresol, 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol in semipermanent hair dyes, however, could lead to skin absorption that would raise the need to assess systemic toxicity.

Such data are available for 6-Amino-*m*-Cresol and 4-Amino-*m*-Cresol, i.e., there are no toxic impurities, the ingredients themselves are not significantly toxic when absorbed into the skin, and there is no reproductive or developmental toxicity or genotoxicity associated with exposure to them. Therefore, it is possible to conclude that 6-Amino-*m*-Cresol and 4-Amino-*m*-Cresol can also be used safely in semi-permanent hair dyes.

Such data are not available to assess the safety of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol for use in semipermanent hair dyes. In this situation, where the ingredients would not be chemically reacted before they are absorbed into the skin, available data do not provide all the information needed. The types of data required for each ingredient include

1. Physical and chemical properties for all ingredients, including the octanol/water partition coefficient
2. Impurities data, especially regarding the presence of *m*-cresol, other organic molecules, and heavy metals
3. Metabolism data, if the metabolism is not similar to that of 4-amino-2-hydroxytoluene and/or *p*-, *m*-, and *o*-aminophenol (ingredients already reviewed by CIR), the following data may be needed:
   a. 28-Day dermal toxicity with histopathology
   b. Dermal reproductive toxicity data
   c. An *in vitro* genotoxicity study for 6-Amino-*o*-Cresol and one genotoxicity study in a mammalian system for 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol; if positive, a 2-year dermal carcinogenicity study using National Toxicology Program methods may be needed.

CONCLUSION

The CIR Expert Panel concludes that the available data support the safety of 6-Amino-*m*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol as used in oxidative and nonoxidative (semipermanent) hair dyes. The available data also support the safety of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol for use in oxidative hair dyes, but are insufficient to support the safety of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol in nonoxidative (semipermanent) hair dyes.

REFERENCES

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

Beilstein File of Organic Compounds. 1998. 6-Amino-m-Cresol, 6-Amino-o-Cresol, and 4-Amino-m-Cresol entries. *Beilstein database*. Columbus, OH: STN International.

Bootman, J. 1984a. 1-Hydroxy-2-amino-5-methylbenzene hemisulphate: Assessment of its capacity to induce genetic damage in *Saccharomyces cerevisiae*. Unpublished data submitted by the Cosmetic, Toiletry, and Fragrance Association (CTFA). 18 pages.[2]

Bootman, J. 1984b. *In vitro* assessment of the clastogenic activity of 1-hydroxy-2-amino-5-methylbenzene hemisulphate in cultured human peripheral lymphocytes. Unpublished data submitted by CTFA.

Bracher, M. et al. 1984. Sister chromid exchange assay on 4-amino-m-cresol using Chinese hamster ovary cells. Unpublished data submitted by CTFA. 19 pages.[2]

Burnett, C. M., and E. I. Goldenthal. 1988. Multigeneration reproduction and carcinogenicity studies in Sprague-Dawley rats exposed topically to oxidative hair-colouring formulations containing *p*-phenylenediamine and other aromatic amines. *Food Chem. Toxicol.* 26:467–474.

Burnett, C., E. I. Goldenthal, S. B. Harris, et al. 1976. Teratology and percutaneous toxicity studies on hair dyes. *J. Toxicol. Environ. Health* 1:1027–1040.

Burnett, C., M. M. Jacobs, A. Seppala, and P. Shubik. 1980. Evaluation of the toxicity and carcinogenicity of hair dyes. *J. Toxicol. Environ. Health* 6:247–257.

Cosmetic, Toiletry, and Fragrance Association (CTFA). 1993. Personal correspondence from GN McEwen.[2]

CTFA. 1999a. Chemical information on 6-Amino-m-Cresol. Unpublished data submitted by CTFA. 29 pages.[2]

CTFA. 1999b. Chemical description on 4-Amino-m-Cresol. Dated Dec 3. Unpublished data submitted by CTFA. 28 pages.[2]

CTFA. 1999c. Concentration of use data on 6-Amino-m-Cresol, 6-Amino-o-Cresol, and 4-Amino-m-Cresol. Dated Mar 11. Unpublished data submitted by CTFA.

CRC Handbook of Data on Organic Compounds. 1998. 6-Amino-m-Cresol and 4-Amino-m-Cresol entries. *HODOC database*. Columbus, OH: STN International.

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

Elder, R. L., ed. 1985. Final report on the safety assessment of *p*-Phenylenediamine. *J. Am. Coll. Toxicol.* 4:203–266.

Elder, R. L., ed. 1988. Final report on the safety assessment of *p*-, *m*- and *o*-Aminophenol. *J. Am. Coll. Toxicol.* 7:279–333.

Elder, R. L., ed. 1989. Final report on the safety assessment of 4-Amino-2-Hydroxytoluene. *J. Am. Coll. Toxicol.* 8:569–587.

European Union. 1995. *Cosmetics Directive of the European Union*. Updated version—Incorporating all amendments until August 1, 1995. Dir. 76/768/EEC, Annex IV.

Fautz, R. 1994. *In vivo/in vitro* unscheduled DNA synthesis in rat hepatocytes with A75 (6-Amino-*m*-Cresol). Unpublished data submitted by CTFA. 30 pages.[2]

Fautz, R., and W. Völkner. 1991a. *In vivo/in vitro* unscheduled DNA synthesis in rat hepatocytes with 4-amino-3-methylphenol in aqua bidest. Unpublished data submitted by CTFA. 1 page.[2]

Fautz, R., and W. Völkner. 1991b. *In vivo/in vitro* unscheduled DNA synthesis in rat hepatocytes with 4-amino-3-methylphenol in DMSO/PEG 400 (1 + 9). Unpublished data submitted by CTFA. 30 pages.[2]

Food and Drug Administration (FDA). 1984. Cosmetic product formulation and frequency of use data. *FDA database*. Washington, DC: FDA.

FDA. 1992. Modification in voluntary filing of cosmetic product ingredient and cosmetic raw composition statements. Final rule. *Federal Register* 57:3128–3130.

FDA. 1998. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.

Forschungs GmbH. 1984a. Three month toxicity study with "Oxyrot," "1-hydroxy-3-methyl-4-aminobenzol-sulfate" [4-Amino-m-Cresol]. Unpublished data submitted by Forschung GmbH. 34 pages.[2]

Forschungs GmbH. 1984b. Teratogenicity study of "Oxyrot," "1-hydroxy-3-methyl-4-aminobenzyl-sulfate" [4-Amino-m-Cresol]. Unpublished data submitted by Forschungs GmbH. 55 pages.[2]

Forschungs GmbH. 1985. Four week toxicity study with "Oxygelb," "1-hydroxy-2 amino-5-methyl-benzyl" [6-Amino-m-Cresol]. Unpublished data submitted by Forschungs GmbH. 36 pages.[2]

Gago-Dominguez, M., J. E. Castelao, J. M. Yuan, M. C., Yu, and R. K. Ross. 2001. Use of permanent hair dyes and bladder-cancer risk. *Int. J. Cancer* 91:575–579.

Gago-Dominguez, M., et al. 2003. Permanent hair dyes and bladder cancer: Risk modification by cytochrome P4501A2 and *N*-acetyltransferases 1 and 2. *Carcinogenesis* 24:483–489.

Goel, R. K., K. P. Kansal, and S. N. Sharma. 1979. Infrared absorption spectra of 6-Amino-m-Cresol. *Indian J. Pure Appl. Phys*. 17:778–780.

Harvison, P. J., A. J. Forte, and S. D. Nelson. 1986. Comparative toxicities and analgesic activities of three monomethylated analogues of acetaminophen. *J. Med. Chem*. 29:1737–1743.

Hazleton Laboratories. 1982. 1-Hydroxy-2-amino-5-methylbenzene: Oral (gavage) teratology study in the rat. Unpublished data submitted by Hazleton Laboratories. 33 pages.[2]

Helzlsouer, K., D. Rollison, and S. Pinney. 2003. Association between hair dye use and health outcomes: Review of the literature published since 1992. Unpublished data submitted by Clairol, Inc.

Henkel KGaA. 1994. 5-Amino-4-chloro-2-methylphenol-hydrochloride: COLIPA-No. A117. Submission I. Toxicological data for 5-Amino-4-chloro-2-methylphenol-hydrochloride submitted by Henkel KGaA. 31 pages.[2]

Henkel KGaA. 1996. 2-Chloro-6-methyl-3-aminophenol hydrochloride: COLIPA - No. A094. Submission I. Toxicological data for 2-Chloro-6-methyl-3-aminophenol hydrochloride submitted by Henkel KGaA. 31 pages.[2]

Holmstroem, M. 1980. Mutagenicity micronucleus assay of 5 compounds and LD_{50} assay. Unpublished data submitted by CTFA. 33 pages[2]

Hong, S. K., M. A. Valentovic, D. K. Anestis, et al. 1996. Nephrotoxicity of 4-amino-2-chlorophenol and 2-amino-4-chlorophenol in the Fischer 344 rat. *Toxicology* 110:47–58.

International Agency for Research on Cancer (IARC). 1993. *IARC monographs on the evaluation of carcinogenic risks to humans*, Vol. 57, 43–118, 159–165. Lyon, France: IARC.

Jacobs, M. M., C. M. Burnett, A. J. Penicnak, et al. 1984. Evaluation of the toxicity and carcinogenicity of hair dyes in Swiss mice. *Drug Chem. Toxicol.* 7:573–586.

James Robinson Ltd. 1998. Specifications and UV absorption data. Unpublished data submitted by CTFA. 6 pages.[2]

King, M. T., and D. Harnasch. 1991. Mutagenicity study of LGH 110583/3 (6-Amino-m-Cresol) with the chromosome aberration test in bone marrow cells of Chinese hamsters *in vivo*. Unpublished data submitted by CTFA. 14 pages.[2]

Leimbeck, R., and W. Grötsch. 1991. *In vivo* micronucleus test of LGH 110583/3 (6-Amino-m-Cresol) in mice. Unpublished data submitted by CTFA. 25 pages.[2]

Martin, C. N. 1983. Study to determine the ability of 1-hydroxy-2-amino-5-methylbenzolhemisulphate to induce mutations at the Na^+/K^+ ATPase and hypoxanthine/guanine-phosphoribosyl transferase loci in mouse lymphoma L5178Y cells using a fluctuation assay. Unpublished data submitted by CTFA. 15 pages.[2]

McGregor, D. B. 1985. 1-Hydroxy-2-amino-5-methyl-benzenehemisulphate: Sister chomatid exchange in rat bone marrow. Unpublished data submitted by CTFA. 33 pages.[2]

Miltenburger, H. G. 1986. Unscheduled DNA synthesis (UDS) *in vitro* of 4-Amino-m-Cresol. Unpublished data submitted by CTFA. 19 pages.[2]

Miltenburger, H. G., and W. Völkner. 1988. Micronucleus test in bone marrow cells of the mouse with Cos 291 (4-Amino-m-Cresol). Unpublished data submitted by CTFA. 27 pages.[2]

Naniwa, S. 1979. Industrial contact dermatitis due to nitro and amino derivatives. 1st report: Mass examinations of a factory. *J. Dermatol.* 6:59–63.

Naniwa, S. 1982. Industrial contact dermatitis due to nitro and amino derivatives. 2nd report: Experimental study of cross sensitivities of amino derivatives. *J. Dermatol.* 9:367–373.

Nishi, H., K. Nakamura, H. Nakai, and T. Sato. 1997. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers. *J. Chromatogr*. 757:225–235.

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

Noser, F. 1979a. Ames test with 6-Amino-m-Cresol. Unpublished data submitted by CTFA. 10 pages.[2]

Noser, F. 1979b. Ames test with 4-Amino-m-Cresol. Unpublished data submitted by CTFA. 10 pages.[2]

Pepe, R. C., J. A. Wenninger, and G. N. McEwen, Jr., eds. 2002. *International cosmetic ingredient dictionary and handbook*, 9th ed., Vol. 1. Washington, DC: CTFA.

Regulated Chemicals Listing. 1998. 6-Amino-m-Cresol, 6-Amino-o-Cresol, 4-Amino-m-Cresol, 5-Amino-6-Chloro-o-Cresol, and 4-Chloro-2-Aminophenol entries. *Chemlist database.* Columbus, OH: STN International.

Son, O. S., D. W. Everett, and E. S. Fiala. 1980. Metabolism of o-methyl-[^{14}C]toluidine in the F344 rat. *Xenobiotica* 10:457–468.

Spectral Database Information System. 1998. 6-Amino-m-Cresol, 6-Amino-o-Cresol, 4-Amino-m-Cresol, and 4-Chloro-2-Aminophenol entries. *Specinfo database*. Columbus, OH: STN International.

Tomoda, A., K. Tomioka, and M. Minami. 1989. Increased concentrations of haemoglobin X and Y in the erythrocytes of workers in a chemical plant in Japan. *Br. J. Indus. Med*. 46:502–504.

Völkner, W., and A. Heidemann. 1991. Micronucleus assay in bone marrow cells of the mouse with LGH 110583/3 (6-Amino-m-Cresol). Unpublished data submitted by CTFA. 27 pages.[2]

Zeiger, E., B. Anderson, S. Haworth, T. Lawlor, and K. Mortelmans. 1988. *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. *Environ. Mol. Mutagen.* 11(Suppl 12):1–158.