Amended Safety Assessment of Acid Orange 3 as Used in Cosmetics

Status:	Draft Amended Report for Panel Review
Release Date:	September 1, 2022
Panel Meeting Date:	September 26-27, 2022

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

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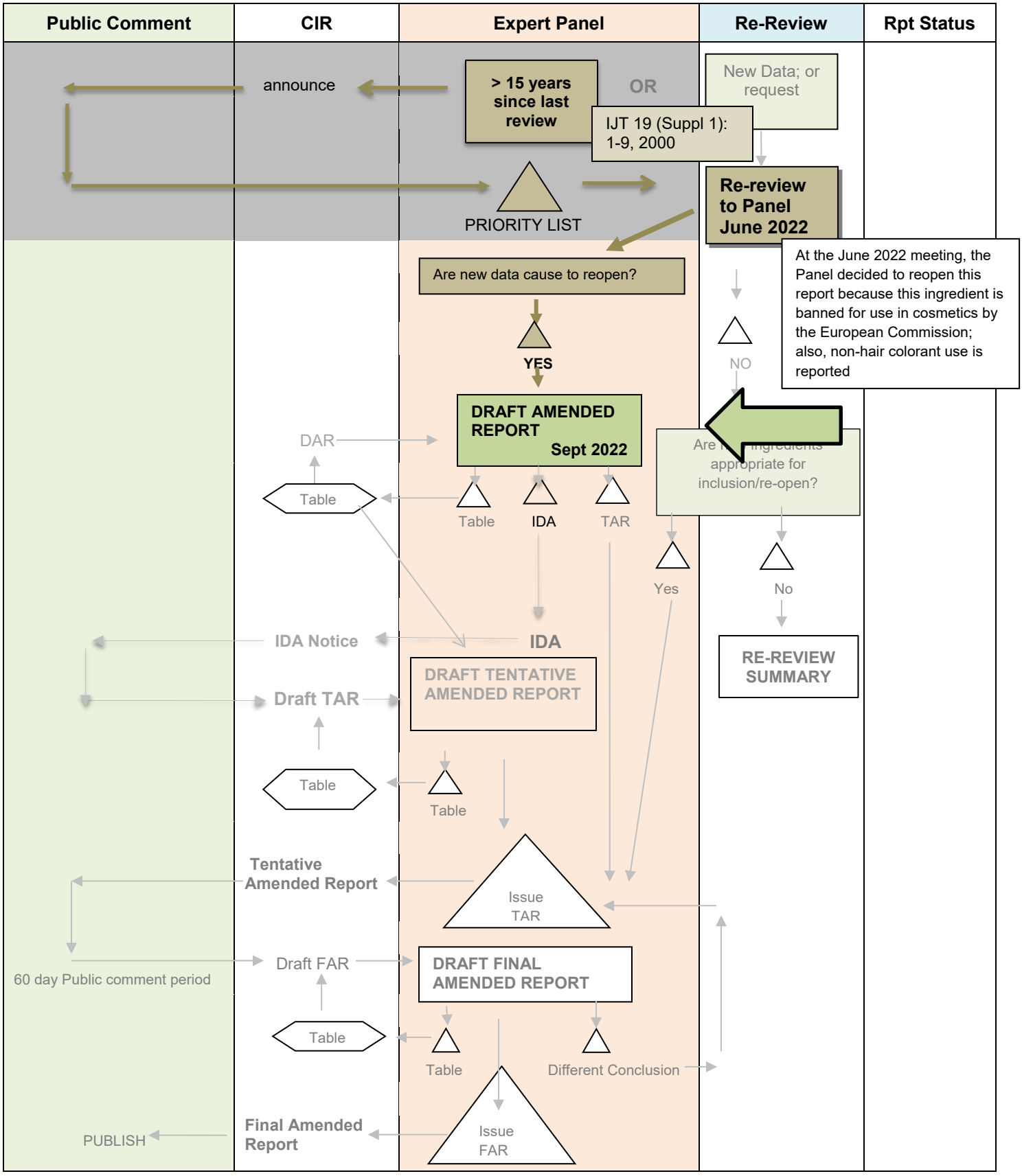
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Acid Orange 3

MEETING September 2022



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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, Senior Scientific Analyst/Writer, CIR
Date: September 1, 2022
Subject: Amended Safety Assessment of Acid Orange 3 as Used in Cosmetics

Enclosed is the Draft Amended Report on the Safety Assessment of Acid Orange 3 as Used in Cosmetics. (It is identified as *report_AcidOrange3_092022* in the pdf document). Acid Orange 3 was previously reviewed by the Panel in a safety assessment that was published in 2000. At that time, the Panel concluded that Acid Orange 3 is safe for use in hair dye formulations at concentrations < 0.2%. In June 2022, the Panel re-opened the safety assessment for this ingredient, which functions as a hair colorant, due to it being banned for use in cosmetics by the European Commission. The Panel also noted reported uses in a non-hair dye cosmetic formulation.

According to 2022 VCRP survey data, Acid Orange 3 is used in one non-hair dye formulation: a nail polish and enamel (*VCRP_AcidOrange3_092022*). The results of the concentration of use survey provided by the Council in 2022 indicate that there are no uses for this ingredient. When the original safety assessment was published in 2000, Acid Orange 3 was reported to be used in 4 hair dye formulations (data acquired in 1997). At that time, concentrations of use were no longer reported by the FDA; however, data available from the FDA in 1984 indicate that Acid Orange 3 was used in one hair dye formulation at a concentration between 10% and 25% and 33 uses were reported at < 1%.

Since the June meeting, no new data have been submitted.

Additional supporting documents for this report package include a flow chart (*flow_AcidOrange3_092022*), report history (*history_AcidOrange3_092022*), a search strategy (*search_AcidOrange3_092022*), the previously published report (*original report_AcidOrange3_092022*), a data profile (*dataprofile_AcidOrange3_092022*), and the minutes from all the past meetings at which Acid Orange 3 was discussed (*transcripts_AcidOrange3_092022*).

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

Acid Orange 3 History

2000– The CIR's Final Report on the Safety Assessment of Acid Orange 3 in the *IJT* after the report was finalized by the Panel in 1997. Based on the available animal and clinical data available at that time, the Panel concluded that Acid Orange 3 is safe for use in hair dye formulations at concentrations < 0.2%.

June 2022 – Review of the available published literature since 2000 was conducted in accordance to CIR Procedures regarding re-review of ingredients after ~15 years. The Panel re-opened the safety assessment for this ingredient, due to it being banned for use in cosmetics by the European Commission. The Panel also noted reported uses in a non-hair dye cosmetic formulation.

Acid Orange 3 Data Profile* – September 2022 – Christina Burnett																														
				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Acid Orange 3	XO	O	O							O	O		O	O	O		O	O					X						

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

Acid Orange 3

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Acid Orange 3	6373-74-6	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Search Strategy (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.

Internet searches using trade names and other technical names. No relevant hits.

LINKS

Search Engines

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

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

Pertinent Websites

- wINCI - http://webdictionary.personalcarecouncil.org
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

DECEMBER 1996 – INITIAL REVIEW

Full Panel – December 16-17, 1996

Dr. Andersen noted that Acid Orange 3 was not included on the agenda for the public session of this Panel meeting. However, during Team reviews (closed session) of this ingredient yesterday, it was determined that the available data are sufficient for arriving at a conclusion on the safety of Acid Orange 3 during the public session.

Dr. Belsito noted that Acid Orange 3 is a coal tar hair dye, and, as such, is regulated under specific provisions of the Federal Food, Drug, and Cosmetics Act of 1938. He said that though dermal irritation and sensitization data are absent from this review, these data are not needed in order for the Panel to arrive at a conclusion on the safety of Acid Orange 3, as long as the product is marketed with a warning statement and patch test instructions as required by law.

Furthermore, Dr. Belsito also said that based on the dermal teratogenicity and dermal carcinogenicity studies on a semi-permanent hair dye formulation containing 0.2% Acid Orange 3, his Team concluded that Acid Orange 3 is safe as used in hair dyes at concentrations up to 0.2%.

The Panel unanimously concluded that Acid Orange 3 is safe as used in hair dyes at concentrations up to 0.2%, and voted in favor of issuing a Tentative Report with this conclusion.

JUNE 1997 – FINAL REPORT

Full Panel – June 5-6, 1997

Dr. Schroeter noted that a Tentative Report was issued at the December 16-17, 1996 Panel meeting, and that no comments were received during the 90-day public comment period. He also noted that the following conclusion on the safety of Acid Orange 3 was approved by the Panel: 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 $\leq$ 0.2%.

Dr. Bailey said that based on the structure of Acid Orange 3, he would predict that it contains impurities such as benzidine 4-aminobiphenylalanine and other free aromatic amines, which would need to be addressed in terms of their risk to the consumer. He noted that, many years ago, FDA banned a dye that is not too dissimilar to Acid Orange 3 because of its aromatic amine content. Furthermore, he noted that benzidine and 4-aminobiphenyl are very potent human carcinogens.

Dr. Shank said that the carcinogenicity of impurities is no longer a concern because of the negative dermal carcinogenicity data on Acid Orange 3 in the Tentative Report.

Dr. Belsito recalled that the Panel's concentration limit of 0.2% is the test concentration of Acid Orange 3 in the two-year skin painting carcinogenicity study.

The Panel voted unanimously in favor of issuing a Final Report with the following 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 $\leq$ 0.2%.

JUNE 2022 PANEL MEETING – RE-REVIEW CONSIDERATION (WITH 2 OTHER HAIR DYE GROUPS)

Belsito's Team Meeting – June 16, 2022

Dr. Belsito - So hair dyes, this is going to take a…. there is more than one hair dye here. I thought we were only going to look at one at a time. What's going on here? Reviewed as a group before.

Monice Fiume (CIR) - They were not, but being that this is the first time groups of reviews have been brought to you are the rereview documents. We were trying to figure out if there were ways to group hair dyes or preservatives or something like that together because they were similar types of functions. But I don't think this was the best example in retrospect.

Dr. Belsito - Yeah. So then let's go through this. So Orange 3 is now been banned in Europe, Acid Orange 3. I think we need to reopen it not only because of that, but in the use section, it says it's used and it's a new product now, which is a nail enamel.

And then the cresols are also, I think, problematic and need to be reopened. Some of them clearly seem to have carcinogenicity activity and I have a note here that seek Council comments and wave 3 of the cresols.

Will this be addressed in the rereview before publication? Let me get to wave 3.

Dr. Snyder - PCPC comments.

Dr. Belsito - Yeah, I'm just. So yeah.

Christina Burnett (CIR) - There was a typo in my memo that they pointed out at the very end. Two of the ingredients. Uh yeah, I think it's two or three of the ingredients that I said were on Annex Two are actually on Annex 3.

Dr. Belsito - Yeah. So this is starts on PDF page 8. I mean I think we need to open up the cresols as well.

Dr. Liebler - I agree.

Dr. Belsito - Because I think the amino position has significant effects on the toxicity. Then back to the next set of hair dyes. It was so it was just cresols and Acid Orange 3, right?

Dr. Liebler - There's one more.

Dr. Belsito - Oh yeah, the N,N Bis 2-Hydroxyethyl p-Phenylenediamine Sulfate. I didn't make any comment on that, so I don't think that I felt it needed to be reopened.

Dr. Liebler - Yeah, I thought it was a do not reopen unless Don identifies a rational rationale having to do with the EU, perhaps. Doesn't look like it, so do not reopen.

Dr. Snyder - Was the nitrosating issue in the original?

Dr. Belsito - It's. Yeah, the so the European Commission further advises this hair dye ingredient is a tertiary, meaning that is prone to nitrosation and should not be used in combination with nitrosating *(inaudible) substances. I guess that is usually read in our discussion, not a conclusion. So you know in the rereview summary where we say we've decided not to reopen it, we can just point that out.

Dr. Liebler - Yeah.

Dr. Belsito - Yeah, I mean the you didn't ban at, they just issued caution when he you know with use.

Dr. Liebler - Right.

Dr. Belsito - And they limited the nitrosamine content should be less than 50 parts per billion.

When?

Dr. Snyder - They asked, well, they also said it was safe up to 2.5%, my notes say.

Dr. Belsito - Yeah. And what is the current use?

Dr. Snyder - I don't know.

Christina Burnett (CIR) - 1.3 is the maximum.

Dr. Belsito - To 1.3 right.

Christina Burnett (CIR) - Yes.

Dr. Belsito - Yeah. So it's well below what the EU restricted. So I don't think we need to reopen it. And then just in the discussion or in some point the document put about the *(inaudible). But the cresols and the Acid Orange 3 I think unfortunately need to be reopened.

Carol Eisenmann (PCPC) - Hi I have one request for the cresols that they could be in the same report but all the data on each cresols I'll be kept together because I think read across as we've said before on these materials is not appropriate. This was done before you started looking at each hair dye individually.

Dr. Belsito - Yeah. I agree, Carol.

Monice Fiume (CIR) - I'm sorry. Carol, can you please repeat what you are, clarify what you said?

Carol Eisenmann (PCPC) - That'd be nice. For all the so you, you can have them in the same report, but like all the data be in for one ingredient be together. So you can see what's the data on that ingredient rather than you know sometimes you're having a paragraph that has all the you summarize all the data, the acute tox data on all of the ingredients in one paragraph make it, you know, separate out. In other words, you the acute chronic reproductive development for one ingredient and then go to the next one and go through the order. In other words, it's going to be like several separate reports. In one report, rather than or make them separate reports because they should not read across isn't appropriate for them.

Dr. Liebler - So.

Yeah, it might be tricky to do that. I mean, one thing that could be done is the endpoint data summary tables could be organized by ingredient.

Dr. Belsito - Right.

Christina Burnett (CIR) - We can do that.

Dr. Liebler - And you could, like I don't mean to dismiss your suggestion, Carol entirely. But the, best way to get an eagle eye view of the data would be those summary tables and that should be, I agree that should be organized by ingredient.

Dr. Belsito - Exactly.

Dr. Liebler - And then you know, whatever Christina. Uh, you know, can come up with in terms of sort of organizing the various tox endpoints in the report text by ingredients to the maximum extent that's possible. I agree that's desirable.

Dr. Belsito - OK. Any other comments on this? Cresols are going to be fun.

Christina Burnett (CIR) - Wait until you see the other two I'm working on.

Dr. Belsito – Oh Lord.

Christina Burnett (CIR) - Sorry.

Cohen's Team Meeting – June 16, 2022

Minutes not captured.

Full Panel Meeting – June 17, 2022

Dr. Bergfeld - OK, we're off to the next set of items, which is other items called Hair Dyes. Doctor Belsito.

Dr. Belsito - OK, so this is not a rereview of one hair dye, that's it's a rereview of several. So we have Acid Orange 3, we have NN, Bis 2 hydroxyethyl paraphenylenediamine sulfate. And then we have the cresols and the amino phenols. And we felt that among this group. We need to reopen Acid Orange 3. We need to reopen the cresol aminophenol group, but we did not need to reopen the NN, Bis 2 hydroxyethyl paraphenylenediamine sulfate.

Dr. Bergfeld - Is there a second on the?

Dr. Cohen - 2nd.

Dr. Bergfeld - Any further discussion regarding which ones will be reopening?

Dr. Belsito - Uh, yeah. So the only discussion really is whether we do the cresol aminophenol group as a whole group, because the actually the positioning of the amino group on the cresol may have significant result in significant differences in the toxicology of the material. It was suggested that by our panel that they all be included in the same report. But that particularly would be presenting the data on toxicity etcetera that instead of as we typically would do like acute oral, you know subchronic chronic that we do that for each. So we do six amino M cresol and then we go through the various oral studies for that. Then we do four amino increase all and do all the tox studies for that so. It will be much clearer in our minds what we have for each of the different materials in this group, because I suspect that we may find that some are safe and some are insufficient. Maybe some should be banned, I don't know, but.

Dr. Bergfeld - I mean. I want to ask Bart about your recommendation.

Dr. Bart Heldreth - I think that sounds perfect. I think that sounds perfectly fine. You know we need to look at all of these one way or another, and it certainly makes complete sense to me to pull these out and make it very clear that they're separate and that there's really no chance for read across between them and that they're individuals. I think that makes perfect sense.

Dr. Bergfeld - OK, how about the orange dye? Anyone want to make a comment on that one? That one is going to be reopened at least this.

Dr. Belsito - Yeah. There's new data and it's just been banned by the EU. So I think we need to look at it.

Dr. Bergfeld - OK.

Dr. Cohen - Yeah, done. We had a lot of deliberation over this and it seems like there's also a paucity of data that may result in from the ban that results in the ban and we had gone back and forth whether this might not be reopened and put into a rereview summary, but I think we came around several times to your team's conclusion.

Dr. Bergfeld - Well, we are then voting on the reopening of the acid orange and we're not reopening the Bis, but also reopen the creosol. Is that right?

Dr. Belsito - Correct.

Dr. Klaassen - Correct.

Dr. Bergfeld - OK, I'm going to call the question then all those opposing. Abstaining. I assume it's unanimous that we're moving forward with reopening of two groups here.

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ABBREVIATIONS

CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Da	Daltons
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook*
FDA	Food and Drug Administration
HPLC	high performance liquid chromatography
IARC	International Agency for Research on Cancer
NR	none reported
Panel	Expert Panel for Cosmetic Ingredient Safety
SCCNFP	Scientific Committee on Cosmetic and Non-Food Products
US	United States
UV	ultraviolet
VCRP	Voluntary Cosmetic Registration Program

INTRODUCTION

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), Acid Orange 3 is reported to function in cosmetics as a hair colorant.[1] Acid Orange 3 was previously reviewed by the Expert Panel for Cosmetic Ingredient Safety (Panel) in a safety assessment that was published in 2000.[2] At that time, the Panel concluded that Acid Orange 3 is safe for use in hair dye formulations at concentrations ≤ 0.2%. In accordance with its Procedures, the Panel evaluates the conclusions of previously-issued reports approximately every 15 years, and it has been at least 15 years since this assessment has been issued. In June 2022, the Panel determined that this safety assessment should be re-opened for re-evaluation due to Acid Orange 3 being banned for use in cosmetics by the European Commission. The Panel also noted reported use in a non-hair dye cosmetic formulation.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties. Excerpts from the summaries of the previous report on Acid Orange 3 are disseminated throughout the text of this re-review document, as appropriate, and are identified by *italicized text.*

CHEMISTRY

Definition and Structure

According to the *Dictionary*, Acid Orange 3 (CAS No. 6373-74-6) is classed chemically as a nitro color that conforms to the structure in Figure 1.[1] This ingredient is an oxidative hair dye ingredient comprising 2 secondary amines.

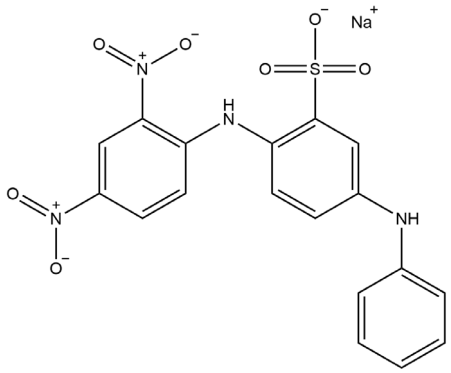

Figure 1. Acid Orange 3

Of concern in cosmetics is the conversion of secondary amines (R1-NH-R2) into *N*-nitrosamines that may be carcinogenic.[3] However, the amine functional groups in Acid Orange 3 are aryl amines. While many secondary amines are readily nitrosated to form isolatable nitrosamines, aryl amines ultimately yield diazonium salts, instead of nitrosamines. Consequently, there is no appreciable concern with regard to *N*-nitrosation of Acid Orange 3.

Chemical Properties

Acid Orange 3 occurs as dark orange-brown microcrystals.[2] It has a formula weight of 452.39 Da and is very soluble in water and ethanol. Acid Orange 3 absorbs in the UV region of the electromagnetic spectrum.

Method of Manufacture

Acid Orange 3 is prepared by the condensation of 1-chloro-2,4-dinitrobenzene with 5-amino-2-anilinobenzenesulfonic acid.[2]

Impurities

Initial analysis of one batch of Acid Orange 3 by ultraviolet light (UV) spectroscopy reported that it contained only 67% Acid Orange 3.[2] 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

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

According to 2022 VCRP survey data, Acid Orange 3 is used in one formulation, it is reported to be used in a nail polish and enamel.[4] The results of the concentration of use survey provided by the Council in 2022 reported no uses for this ingredient.[5] When the original safety assessment was published in 2000, Acid Orange 3 was reported to be used in 4 hair dye formulations (data acquired in 1997).[2] At that time, concentrations of use were no longer reported by the FDA; however, data available from the FDA in 1984 indicated that Acid Orange 3 was used in one hair dye formulation at a concentration between 10% and 25%,and 33 hair dye formulations at $\leq$ 1%.

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

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

Product labels shall also bear patch test instructions for determining whether the product causes skin irritation. However, whether or not patch testing prior to use is appropriate is not universally agreed upon. The Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 h after application of the test material and prior to the use of a hair dye formulation. Conversely, a report in Europe suggests that self-testing has severe limitations, and may even cause morbidity in consumers.[6,7] Hair dye products marketed and sold in the US, though, must follow the labeling requirements established by the Food, Drug, and Cosmetic Act.

In the European Union, the Scientific Committee on Cosmetic and Non-Food Products (SCCNFP) determined that Acid Orange 3 could not be considered safe for hair dying purposes due to the lack of an adequate safety dossier.[8] Under European regulations for cosmetic ingredients, Acid Orange 3 is categorized in Annex II, the list of substances prohibited in cosmetic products in Europe.[9]

Non-Cosmetic

Acid Orange 3 is used in textile dyes.[2]

TOXICOKINETIC STUDIES

Toxicokinetics studies were not found in the published literature, and unpublished data were not submitted.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Acute toxicity studies were not found in the published literature, and unpublished data were not submitted.

Short-Term, Subchronic, and Chronic Toxicity Studies

In short-term oral toxicity studies, adverse effects were not observed in rats dosed with $\leq$ 1500 mg/kg or mice dosed with $\leq$ 1000 mg/kg Acid Orange 3 in corn oil for 14 d.[2] *In a 13-wk dermal study, adverse effects were not observed in rabbits dose twice weekly with 1 ml/kg of undiluted hair dye formulation containing 0.2% Acid Orange 3. Acid Orange 3 in corn oil was tested at gavage doses of 94 - 1500 mg/kg in rats and at doses of 62 - 2000 mg/kg in mice for 13 wk. Lesions of the kidneys were observed in rats dosed with 1500 mg/kg and in mice dosed with 1000 or 2000 mg/kg. In a 24-mo chronic oral toxicity study, dogs were fed 19.5 or 97.5 mg/kg/d hair dye formulation with 0.24% Acid Orange 3: no adverse effects were observed.*

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Teratogenic and embryotoxic effects were not observed in a dermal study in which gravid rats received 2 ml/kg applications of a hair dye formulation containing 0.2% Acid Orange 3 every third day of gestation (up to day 19), and reproductive effects were not observed in a multigeneration study in which rats received up to 0.5 ml topical applications of a hair dye formulation containing 0.2% Acid Orange 3 twice weekly.[2] In oral studies with a hair dye formulation containing 0.24% Acid Orange 3, teratogenic or reproductive effects were not observed in female rabbits that received 19.5 or 97.5 mg/kg/d on gestation days 6 - 18, in female rats that received 1950 or 7800 ppm on gestation days 6 – 15, or in male and female rats that received 1950 or 7800 ppm 8 wk prior to mating, during gestation, and during 21 d of lactation.

GENOTOXICITY STUDIES

Acid Orange 3, tested at concentrations of ≤ 2000 µg/plate in dimethyl sulfoxide, was mutagenic to Salmonella typhimurium in a preincubation test, with and without metabolic activation.[2] Acid Orange 3 was active in a transformation assay without metabolic activation using BALB/c-3T3 cells when tested at concentrations ≤ 0.222 mM.

CARCINOGENICITY STUDIES

In a dermal carcinogenicity study in which rats received twice weekly 0.5 ml applications of a hair dye formulation containing 0.2% Acid Orange 3 for a year, "possibly compound related effects" were observed , including enlarged and/or firm livers and an increase in para-thyroid gland hyperplasia, hepatocellular hypertrophy or hyperplasia, hyperkeratosis and dermatitis, and hyperkeratosis and/or acanthosis involving the gastric mucosa.[2] A carcinogenic effect was not observed for mice used in a 23-mo 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 in corn oil, 5 d/wk for 103 wk, clear evidence of carcinogenic activity was observed for female rats that received 750 mg/kg 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 in all dose groups.

The International Agency for Research on Cancer (IARC) determined that Acid Orange 3 is not classifiable as to its carcinogenicity to humans (Group 3).[10] This determination was based on inadequate evidence in humans and limited evidence (previously summarized; see above) in experimental animals for carcinogenicity of Acid Orange 3.

OTHER RELEVANT STUDIES

Cytotoxicity

Acid Orange 3 was cytotoxic to BALB/c-3T3 cells in tissue culture.[2] On average, the concentration that allowed a 50% survival was 0.102 mM.

DERMAL IRRITATION AND SENSITIZATION

Dermal irritation and sensitization data were not found or submitted at the time of the original report.

Sensitization

Animal

In a modified Buehler open epicutaneous test, 10 albino guinea pigs were induced with 10% Acid Orange 3 (0.1 ml) in propylene glycol on shaved left flanks 3 times weekly for 3 consecutive weeks.[11] The test site was a 1.8 cm circular area. Test sites were observed for dermal reactions 24 h after the last application. Following a non-treatment period of 2 wk, the animals received challenge applications of 2.5%, 5%, and 10% Acid Orange 3 on the shaved right flank. After 24 h, the animals were depilated to observed dermal reactions. Positive reactions were observed with all 3 challenge concentrations, with 30% of the animals having a positive reaction at 2.5%, 60% at 5%, and 80% at 10%. Additional testing with challenge concentrations of 1% or less was negative. The positive control (0.5% 2,4-dinitrochlorobenzene) yielded expected results.

OCULAR IRRITATION STUDIES

Ocular irritation studies were not found in the published literature, and unpublished data were not submitted.

HAIR DYE EPIDEMIOLOGY

Hair dyes may be broadly grouped into oxidative (permanent) and direct (temporary or semi-permanent) dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes consist of preformed colors. Acid Orange 3 is reported to be used in semi-permanent and oxidative hair dye formulations. While the safety of individual hair dye ingredients is not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer. A detailed summary of the available hair dye epidemiology data is available at https://www.cir-safety.org/cir-findings.

SUMMARY

Acid Orange 3 is reported to function in cosmetics as a hair colorant. Acid Orange 3 was previously reviewed by the Panel in a safety assessment that was published in 2000. At that time, the Panel concluded that Acid Orange 3 is safe for use in hair dye formulations at concentrations ≤ 0.2%. In accordance with its Procedures, the Panel evaluates the conclusions of previously-issued reports approximately every 15 years, and it has been at least 15 years since this assessment has been issued. In June 2022, the Panel determined that this safety assessment should be re-opened for re-evaluation due to Acid Orange 3 being banned for use in cosmetics by the European Commission. The Panel also noted reported use in a non-hair dye cosmetic formulation.

According to 2022 VCRP survey data, Acid Orange 3 is used in one formulation, it is reported to be used in a nail polish and enamel. The results of the concentration of use survey provided by the Council in 2022 reported no uses for this ingredient. When the original safety assessment was published in 2000, Acid Orange 3 was reported to be used in 4 hair dye formulations (data acquired in 1997). At that time, concentrations of use were no longer reported by the FDA; however, data available from the FDA in 1984 indicated that Acid Orange 3 was used in one hair dye formulation at a concentration between 10% and 25%,and 33 hair dye formulations at ≤ 1%.

In the European Union, the SCCNFP determined that Acid Orange 3 could not be considered safe for hair dying purposes due to the lack of an adequate safety dossier. Under European regulations for cosmetic ingredients, Acid Orange 3 is categorized in Annex II, the list of substances prohibited in cosmetic products in Europe.

The IARC determined that Acid Orange 3 is not classifiable as to its carcinogenicity to humans (Group 3). This determination was based on inadequate evidence in humans and limited evidence in experimental animals for carcinogenicity of Acid Orange 3.

In a modified Buehler open epicutaneous test in guinea pigs, positive reactions were observed in skin that was induced with 10% Acid Orange 3 and challenged with 2.5%, 5%, and 10% Acid Orange 3. Additional testing with challenge concentrations of 1% or less was negative.

The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer.

No toxicokinetics studies or ocular irritation studies on Acid Orange 3 were found in the published literature, and unpublished data were not submitted.

PREVIOUS 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.[2] *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 Panel recognizes that irritation and sensitization data on Acid Orange 3 are absent from this report. However, 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 Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures.[2]

DISCUSSION

To be determined.

CONCLUSION

To be determined.

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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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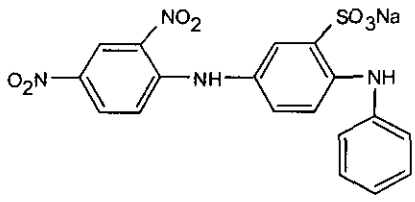

FIGURE 1
Chemical structure of Acid Orange 3.

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%.

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2022 FDA VCRP Raw Data

ACID ORANGE 3	Nail Polish and Enamel	1