Amended Safety Assessment of 4-Amino-*m*-Cresol as Used in Cosmetics

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

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

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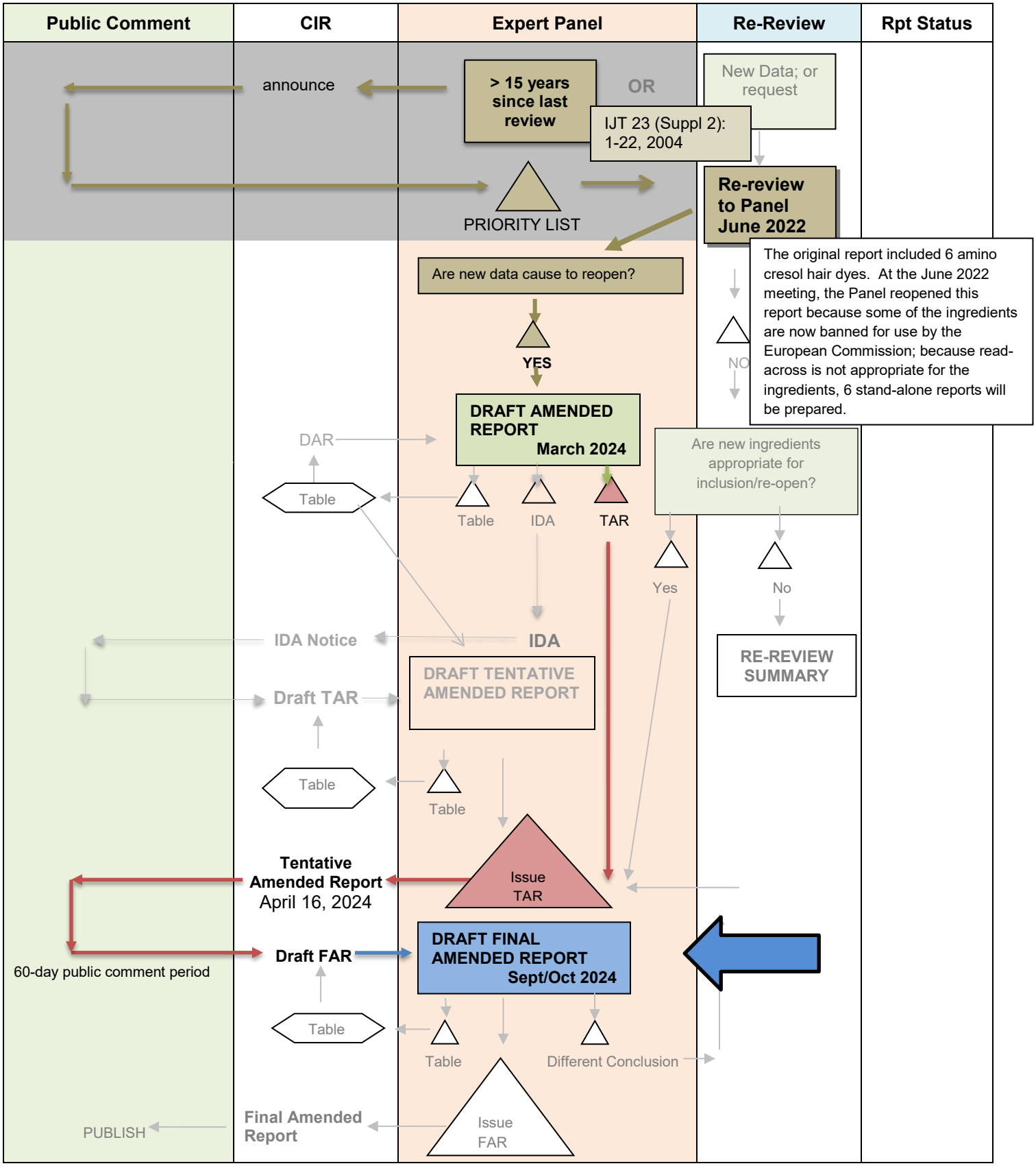
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY 4-Amino-*m*-Cresol

MEETING September/October 2024



Cosmetic Ingredient Review

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Memorandum

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

Enclosed is the Draft Final Amended Report on the Safety Assessment of 4-Amino-*m*-Cresol as Used in Cosmetics. (It is identified as *report_4-Amino-m-Cresol_092024* in the pdf document). At the March 2024 meeting, the Panel issued a Tentative Amended Report with the conclusion that 4-Amino-*m*-Cresol is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

No additional data have been received for this report. Comments provided by the Council on the Tentative Amended Report have been addressed (*PCPCcomments_4-Amino-m-Cresol_092024* and *response-PCPCcomments_4-Amino-m-Cresol_092024*).

Other supporting documents for this report package include the original report (*orginalreport_4-Amino-m-Cresol_092024)*, a flow chart (*flow_4-Amino-m-Cresol_092024*), report history (*history_4-Amino-m-Cresol_092024*), a search strategy (*search_4-Amino-m-Cresol_092024*), a data profile (*dataprofile_4-Amino-m-Cresol_092024*), transcripts from the meeting at which this amended report was discussed (*transcripts_4-Amino-m-Cresol_092024*), and the minutes from all the meetings at which 4-Amino-*m*-Cresol was discussed during the original review (*originalminutes_4-Amino-m-Cresol_092024*).

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

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Alexandra Kowcz, MS, MBA
Industry Liaison to the CIR Expert Panel

DATE: April 23, 2024

SUBJECT: Tentative Report: Amended Safety Assessment of 4-Amino-m-Cresol as Used in Cosmetics

The Personal Care Products Council respectfully submits the following comments on the Tentative report, Amended Safety Assessment of 4-Amino-m-Cresol as Used in Cosmetics.

Method of Manufacture – The CIR report on Coal Tar (reference 7) does not seem to be an appropriate reference, specifically for the following sentence: "However, non-amino-substituted cresols are traditionally obtained via distillation of coal tar."

Perhaps these patents for producing 4-Amino-m-Cresol may include useful information on method of manufacture: https://patents.google.com/patent/JP2012062252A/en, https://patents.google.com/patent/CN103508908A/en.

Dermal Absorption, Animal – In the description of dermal absorption in PVG rats it says: "The treated skin was covered with an occlusive plaster during the exposure period. During the exposure time the treated skin area was securely sealed by an occlusive plaster." Since these sentences are saying the same thing, only one of them is needed.

ADME, In Vitro – In the following, only the second "data" is needed: "extrapolating ~~data~~ from rat data to humans in terms of liver metabolism"

Genotoxicity, In Vivo – In the description of the micronucleus assay in mice, it would be helpful to add that bone marrow cells were examined.

Discussion – Since there is only one ingredient in this report, the following needs to be revised: "for these ingredients"

Discussion – Does the Expert Panel want to mention the risk assessment calculations in the Discussion?

Table 2 – In the description of the mammalian cell gene mutation assay, cytotoxicity is mentioned in the results for test 1 (without metabolic activation). Was cytotoxicity observed in tests 2 and 3 (with metabolic activation)?

Table 3 – In the first guinea pig sensitization test, fourth column, please delete “group” in “10 guinea pigsgroup”

4-Amino-*m*-Cresol – September 2024 – Christina Burnett	
Comment Submitter: Alexandra Kowcz, PCPC **Date of Submission: April 23, 2024**	
Comment	**Response/Action**
Method of Manufacture – The CIR report on Coal Tar (reference 7) does not seem to be an appropriate reference, specifically for the following sentence: "However, non-amino-substituted cresols are traditionally obtained via distillation of coal tar." Perhaps these patents for producing 4-Amino-m-Cresol may include useful information on method of manufacture: https://patents.google.com/patent/JP2012062252A/en, https://patents.google.com/patent/CN103508908A/en.	Per Dr. Bart Heldreth, this reference is appropriate. The Panel has stated that CIR should not cite patents. See the transcripts from the March 2024 meeting.
Dermal Absorption, Animal – In the description of dermal absorption in PVG rats it says: "The treated skin was covered with an occlusive plaster during the exposure period. During the exposure time the treated skin area was securely sealed by an occlusive plaster." Since these sentences are saying the same thing, only one of them is needed.	Deleted extra sentence.
ADME, In Vitro – In the following, only the second "data" is needed: "extrapolating data from rat data to humans in terms of liver metabolism"	Deleted extra "data".
Genotoxicity, In Vivo – In the description of the micronucleus assay in mice, it would be helpful to add that bone marrow cells were examined.	Added suggestion.
Discussion – Since there is only one ingredient in this report, the following needs to be revised: "for these ingredients"	Edited to "this ingredient".
Discussion – Does the Expert Panel want to mention the risk assessment calculations in the Discussion?	Additional description of the MOE calculations provided.
Table 2 – In the description of the mammalian cell gene mutation assay, cytotoxicity is mentioned in the results for test 1 (without metabolic activation). Was cytotoxicity observed in tests 2 and 3 (with metabolic activation)?	No further information on cytotoxicity was provided in the source reference.
Table 3 – In the first guinea pig sensitization test, fourth column, please delete "group" in "10 guinea pigsgroup"	Correction made.

4-Amino-*m*-Cresol History

2004– The CIR's 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 was published in the *IJT* after the report was finalized by the Panel in 2000. Based on the available animal and clinical data available at that time, the Panel concluded that 4-Amino-*m*-Cresol is safe as used in oxidative and non-oxidative (semi-permanent) hair dyes.

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 some of the ingredients being banned for use in cosmetics by the European Commission (this ingredient was not one of those banned).

March 2024 - The Panel issued a Tentative Amended Report for public comment with the conclusion that 4-Amino-*m*-Cresol is safe for use as a hair dye ingredient in the present practices of use and concentration described in the safety assessment. This Panel previously reviewed this ingredient as part of a larger group of amino cresol hair dyes; however, because the Panel determined that data for these amino cresol hair dye ingredients could not be read-across the group, re-reviews of each hair dye included in that original 2004 report will now be presented as individual stand-alone reports.

4-Amino-*m*-Cresol Data Profile* – September 2024 – Christina Burnett																														
				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
4-Amino-*m*-Cresol	X O		X O	X	X	X		X			O			O	X O	X O			X	X			X			X	X		

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

4-Amino-*m*-Cresol

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
4-Amino-*m*-Cresol	2835-99-6	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Search (from 2002 on)

PubMed

4-Amino-m-Cresol
((“4-Amino-m-Cresol”) OR (2835-99-6[EC/RN Number])) – 6 hits; 0 relevant

ECHA

Entry for CAS# 2835-99-6 resulted in finding a dossier for “4-amino-m-cresol”.

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

JUNE 2022 PANEL MEETING – RE-REVIEW CONSIDERATION (WITH SEVERAL OTHER HAIR DYES)

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.

Dr. 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 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?

Dr. 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 6-amino-m-cresol and then we go through the various oral studies for that. Then we do 4 amino cresol 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 (CIR)- 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.

MARCH 2024 PANEL MEETING – DRAFT AMENDED REPORT

Belsito's Team Meeting – March 28, 2024

DR. BELSITO: Okay. Then we're going to move onto 4-Amino-*m*-Cresol. Then, again, they had a PCP Wave 2 comment on that document. I was fine with all the, I mean, editorial comments and -- et cetera from PCPC. Was everyone else okay with their comments before we move onto the document?

DR. SNYDER: Well, the Wave 2, they had the comment about the 50 percent absorption used in the margin of safety calculation. Did you want to discuss that?

DR. BELSITO: Yeah. I mean, I agreed with them that the reason for using the 50 percent dermal absorption, an assumption that needs to be corrected in the Summary. I mean, what did you think? I mean, it's excessive, but it's conservative.

DR. SNYDER: That's what I thought. It was just ultra conservative. I agree with that.

DR. BELSITO: Just --

DR. KLAASSEN: I personally do not like the 50 percent. I mean, we have data on this chemical, much better than most chemicals, in regard to absorption. I think we should use, as was suggested, a much lower number. I mean, when you got the data, use the data.

DR. BELSITO: I thought you were going to say that, Curt.

DR. KLAASSEN: What?

DR. BELSITO: I mean, I'm good with using the data too. I can go either way. Allan?

DR. RETTIE: Yeah, I agree with Curt.

DR. BELSITO: Okay.

DR. KLAASSEN: Unfortunately, I couldn't hear you. You were cutting out.

DR. BELSITO: Allan said he agrees with you, Curt.

DR. RETTIE: I'm with you, Curt.

MS. BURNETT: You would like us to rework the calculation using the data from the dermal absorption section?

DR. BELSITO: Yes.

MS. BURNETT: Okay.

DR. KLAASSEN: Yes.

DR. BELSITO: Paul, you're okay with that change?

DR. SNYDER: Yes, I'm fine with that.

DR. BELSITO: Okay. Okay. Then this is really the first time we're looking at this report because it was grouped together with a lot of other hair dye cresols that we now feel we can't read across from. Looking at all of the data, basically, I had a bunch of editorial comments. I, in the end, thought that this was safe as used based upon our calculations. To go through the document, I think that we have one -- I mean, not that we've not done this before, but this is banned in the EU. Is that correct?

MS. BURNETT: No, this one is not.

DR. BELSITO: It is not. Okay.

DR. SNYDER: That's the pyrol -- the next one, pyrogallol.

DR. BELSITO: Oh, okay. Yeah.

MS. BURNETT: This one --

DR. RETTIE: Yeah, it's just in (audio skip) three, which restricts the concentration of use to one and a half percent.

DR. BELSITO: Right. That's right, but our use concentration is within that range.

MS. BURNETT: Right.

DR. SNYDER: 0.14 percent max. It's well below it, yeah, well below it.

DR. BELSITO: Right. Okay. Christina, in the absorption, distribution, metabolism, and excretion section -- and I don't know why I can't see my PDF numbers here.

DR. SNYDER: Page 19.

DR. BELSITO: Okay. Under the in vitro studies in the last paragraph, it talks about analyzing propranolol and you have vinblastine and ranitidine. I think you mean vinplastine. No?

MS. BURNETT: Possibly. I'll go back and check the reference.

DR. BELSITO: Yeah, check the reference because I've never heard of vinplastine. Vinblastine is a chemotherapeutic agent.

MS. BURNETT: Okay.

DR. BELSITO: Under the previous discussion (audio skip) carry over into our discussion, or you just put that up there for reference?

MS. BURNETT: It's there for your reference for this review. It will disappear once you create the new discussion.

DR. BELSITO: Okay. I thought, in the discussion, we should point out the margin of safety study for the oral, the negative DART in genotox, the good dermal and ocular irritation and, actually, dermal sensitization at concentration of use, which is unusual for a hair dye, and that it's not been evaluated for airbrush and safe as used for the conclusion. That was all I had in the discussion. Curt? Paul? Allan?

DR. SNYDER: I didn't have any additions to that. I just thought, yeah, everything is here. We just need to kind of update it.

DR. RETTIE: Yeah, I have no additions. It was a relatively straightforward hair dye, kind of unusual for us.

DR. BELSITO: Curt?

DR. KLAASSEN: It's fine.

DR. BELSITO: Okay. Then we'll move onto pyrogallol.

DR. RETTIE: Oh, I'm sorry. Could we just -- I had a minor comment on the last one on method of manufacture. We never bother about the lack of information, it seems.

DR. BELSITO: The last one meaning lanolin? Or amino-cresol?

DR. RETTIE: The cresol, sorry.

DR. BELSITO: Go ahead.

DR. RETTIE: Yeah, just wanted to get a little bit of clarification on the lack of method of manufacture for these compounds. We rarely have a method of manufacture, and we don't worry about it too much, it seems, because it's nearly always come from a coal tar distillation. I actually found a patent process for production for amino-meta-cresol. I didn't know if we wanted to go to including those types of things, which may be popping up a little bit more, as a reference for how to obtain this, unless, for cosmetic use, it's always this traditional way of obtaining it from manufacturers who've got it from the distillation of coal tar. This was just an unusual one because there was a patent out there for its synthesis for an amino-meta-cresol. Do we know if any chemical synthesis processes are being used these days for coal tar dyes? I don't.

Well, it sounds like we are where we were so we don't need to spend any more time on that.

MS. FIUME: Allan, I just want to --

DR. RETTIE: I'll just send you the -- yeah.

MS. FIUME: I was going to say, typically, we do not use patents, but it's the purview of the Panel if you want that brought in or not. That is totally up to you.

DR. RETTIE: I don't mind either way. It just goes back to discussion about data and really old synthetic methods in some of the products when we carry over information from previous reports. It's maybe not a conversation for now. I'm happy with it as it is.

DR. BELSITO: Monice has raised a question whether we include patent information for method of manufacture. We're told traditionally we haven't done that. I'm fine with following tradition. Curt? Paul?

DR. KLAASSEN: Yeah, I would go without it. If we knew for -- especially, we don't know exactly if that's what they're using for the cosmetic. I'd just leave it out.

DR. SNYDER: I agree.

DR. RETTIE: Yep. Fair enough.

DR. BELSITO: Okay. Anything else on the 4-Amino-m-Cresol? Okay.

Cohen's Team Meeting – March 28, 2024

DR. COHEN: All right. Let's move onto 4-Amino-*m*-Cresol. So, we re-reviewed the safety of a group of cresols, and we reopened them in 2022 largely because some of these hair dyes were being banned for use by the European commission so we've seen them individually. And so, for 2023 the VCRP survey data shows 4-Amino-*m*-Cresol has 28 reported uses in hair dyes and the Council had its survey in 2021 with a max concentration of 0.082 to 0.14 percent in hair dyes and colors. Since the June 2022 meeting no new data has been submitted. We got some Wave 2 comments, and we have a margin of safety. So, any comments or thoughts or needs from here?

DR. ROSS: Susan, your go.

DR. TILTON: I mean, in terms of needs I noted that there is no method of manufacturing, but it seems like that had been previously or nothing was submitted for that. The only other thing I noticed is there was also not a use table was that not typically included for hair dye? I can't remember.

MS. BURNETT: Since it's a single ingredient with only usually one use category we don't create a use table for those.

DR. TILTON: Okay.

DR. COHEN: Its use is hair dye, right. So, I guess --

DR. TILTON: So, I just noted low dermal absorption and rapid metabolism in vitro and in rats. Mixed results for genotoxicity. Not mutagenic in multiple Ames and mammalian gene mutation tests with and without metabolic activation. Not mutagenic in mouse lymphoma assay. Some ambiguous results from micronucleus test in vitro and unscheduled DNA synthesis in vitro and in vivo at higher concentrations. Not genotoxic in vivo in mammalian bone marrow micronucleus tests in rat, liver, and scheduled DNA synthesis tests.

Found to be non-irritant and non-corrosive in EpiSkin and EpiDerm assays with human hair keratinocytes. Non-sensitizing in a guineapig maximization test up to three percent. Minimal ocular irritation up to 1.5 percent in chicken eye tests and also use with guineapigs. The margin of safety for 1.5 percent in formulation with hydrogen peroxide was calculated to be 124 although as noted in the Wave 2. This was a very conservative estimate assuming 50 percent dermal absorption.

Noted earlier that there is really low dermal absorption. I didn't have any noted insufficiencies and concluded with recommend safe as used in hair dyes under oxidative conditions under current concentrations.

DR. COHEN: David, anything else?

DR. ROSS: No, that's a good summary and --

DR. COHEN: Really good.

DR. ROSS: -- I would just comment that it's E.U. Annex III max concentrations up to mixing -- that recommendation is after mixing with hydrogen peroxide not greater than 1.5 percent. As Susan said, the skin absorption's low. Genotox is generally negative and particularly the in vivo was negative. There were some mixed results as they usually are. The DART I had no issues with. The tox, no issues.

I thought maybe the hemisulfate -- I think that came up in the Wave 2 or some other comments -- could be described in the chemistry section. Just a line particularly as that was one of the positive in vivo genotox results with the hemisulfate. With

respect to the margin of safety, yeah, we did it very conservative. I mean, Jinqiu did a very conservative one there. We don't have absorption at 0.14 percent but, as Alex pointed out, we do have absorption at 0.5 percent which I think was about 0.06 percent or 0.068 percent. So, it just depends on how conservative you want to go.

If you want to add in another MOS here using that 0.5 percent concentration you can, but I don't think it's needed since you've been incredibly conservative, and you've come out with a margin of safety of 512 anyway.

DR. COHEN: Yeah. Didn't they use a 50 percent absorption?

DR. ROSS: Yeah, well that's the default in the SCCS notes. Yeah, if you don't have the exact concentration then you use 50 percent.

DR. EISENMANN: I think you can use 50 percent, but I don't like the statement that if you don't have data at the exact concentration -- you don't need the data at the exact concentration to do it. That's what bothers me more. Not that you use 50 percent, it bothers me that the reason is because you don't have data at 0.14.

DR. COHEN: But we do have it.

DR. EISENMANN: There are data that are close enough to 0.14 that are relevant that you could use. You chose to use a conservative approach, that's fine. You don't necessarily need to say because there wasn't data, you just chose to use a conservative approach.

DR. COHEN: I agreed with your comment, Carol. I thought that was right on. So, should we adjust the MOS?

DR. ROSS: I think you should put in another one rather than adjusting because I think that's what SCCS is saying.

DR. COHEN: What absorption?

DR. ROSS: Well, the additional absorption that Carol's pointing out was a study done at 0.5 percent and the absorption was 0.068 percent. As Susan pointed out, and I made the comments, this thing isn't absorbed very readily anyway. So, it's much more accurate doing that so you could certainly add that. I'm sure the MOS will come out looking a lot better but it's just a question of how many you want to add in there. You can certainly prepare.

DR. COHEN: Well, you put one in at what, one percent?

DR. ROSS: No, you put one in at 0.5 percent which is the closest concentration above the 0.14 percent maximum use that we have. So, you'd use the 0.5 percent and then the absorption quantified in that study, I believe, just have in my notes here is 0.068 percent. And so, you can add two or three lines to the margin of safety with a note saying this can be done with the 0.5 percent and the adjusted MOS when using that calculation is XXX.

DR. COHEN: Okay. We can bring that up tomorrow.

DR. BERGFELD: Can I ask a question? And that is the use in oxidative and non-oxidative dyes. I thought it could be used in either one.

DR. ROSS: Yeah. It certainly seems to be used as an oxidative and that was certainly the E.U. Annex III conclusion. Susan in her conclusion said safe as used at 0.14 percent as an oxidative hair dye. I would support that as well, I think. You could probably get away without the clarification because we know in practice 0.1 is going to be used with peroxide. We know 0.2 has got a very low absorption and we know three, it's got a very low toxicity.

But still I think it's good practice in these to define how it's being used, and I think that's what I would suggest as well.

DR. TILTON: That was also a consideration in the MOS calculation.

DR. ROSS: Yeah.

DR. COHEN: That was oxidized. Okay.

DR. BERGFELD: Did you want to discuss the discussion? I mean, you have the previous discussion they were talking about dyes, coloring, you want to keep that?

DR. COHEN: Yeah. I don't see any reason to not keep it. I'm just going to read through it again.

DR. BERGFELD: Right. But nothing to add to it? Do you want to add that margin of safety?

DR. COHEN: That the margin of safety is for oxidized product?

DR. BERGFELD: Mm-hmm.

DR. ROSS: It's certainly is for the SCCP one. I don't know about the one Jinqiu -- I think, I mean, that's how it's going to be used so you may as well define it as being used as an under oxidative conditions because that's how the use is defined.

DR. COHEN: Hold on one second. Okay. Must not exceed 1.5 percent after mixing under oxidative conditions.

MS. BURNETT: That's the SCCP calculation.

DR. ROSS: I didn't come across it under non-oxidative conditions, but I may have missed that. If someone could point me to that.

DR. COHEN: Did anyone see that?

DR. BERGFELD: We have it our previous discussion.

DR. COHEN: Our original report is safe as used in both oxidative and non-oxidative.

DR. BERGFELD: Non oxidative is more of a layering agent than a penetrating agent.

DR. ROSS: As I said, I don't think it'd be an issue either since you've got low absorption of the toxicity but certainly that initial margin of safety that was done by SCCP was done with hydrogen peroxide, I think. If I'm not mistaken, let me just swing back to that.

DR. HELDRETH: Yes, it says so on PDF page 22 last full paragraph.

DR. ROSS: Just got there, Bart.

DR. COHEN: What does it say?

DR. HELDRETH: It says, the SCCP calculated the margin of safety for 1.5 percent for amino-*m*-cresol in a formulation with hydrogen peroxide therefore they're talking about an oxidative hair dye formulation.

DR. ROSS: Could they actually have, you know, the defined absorption rate right there at 41.4 micrograms per centimeter squared but we've got a very high absorption default in the CIR calculation, as Carol pointed out, that still yielded 512.

DR. COHEN: So, this could come through tomorrow safe as used in hair dye.

DR. ROSS: I suspect that's the way it would be, but the question is what is our recommendation? Safe as used as a component of oxidative hair dyes or as hair dyes? And Wilma brought up the question, I'm not sure we've answered that yet.

DR. COHEN: No, no. We have not answered it. I'm getting feedback from you that you're comfortable with it as a non-oxidative hair dye if we need it to be there but maybe I'm overreaching.

DR. ROSS: Maybe you're overreaching.

DR. EISENMANN: I don't think you need it to be there, I think it's just an oxidative hair dye.

DR. ROSS: I think it's an oxidative hair dye thank you, Carol. So that was my point, the use says it's an oxidative hair dye and I think that's where we should stick it.

DR. EISENMANN: Yes.

DR. BERGFELD: Then you should say it in the discussion.

DR. ROSS: Yeah.

DR. COHEN: Well, we can conclude it. Why can't we --

DR. BERGFELD: But we don't need it in the conclusion. You can just put it into the discussion because you're going to say as used and it's as used in oxidative as best we could tell.

DR. ROSS: In the previous dyes we've used have we not concluded that in the conclusion, Wilma?

DR. COHEN: We sometimes have, haven't we?

DR. BERGFELD: We have. I think it's maybe sometimes.

MS. BURNETT: The older reports will say but I think in more recent times we just say in hair dyes.

DR. COHEN: Okay. Fine. I'm okay with that.

DR. ROSS: I still like oxidative, but there you go.

DR. COHEN: I don't have any issue with it. Let's see who's -- Don's doing that one tomorrow.

DR. ROSS: Yeah.

DR. HELDRETH: He is.

MS. BURNETT: I can reiterate that it's used in oxidative hair dyes in the discussion before the conclusion so that its use is reiterated.

DR. COHEN: Okay.

DR. ROSS: All right. That was Wilma's point. That's good. Okay, thank you.

Full Panel Meeting – March 29, 2024

DR. BELSITO: Yeah, so we previously reviewed 6-amino-*m*-cresol along with a lot of amino cresols back in whenever. And subsequently in June 2022, we decided to reopen these ingredients and felt that we really could not read across from *m*-cresols to *o*-cresols to other types, and so we reopened these in various groups. So, this is really the first time that we're looking at this document since reopening it. And based upon the information that we've reviewed, we thought we could go with safe as used as a hair dye for this.

DR. COHEN: Second.

DR. BERGFELD: Any further discussion?

DR. BELSITO: In the discussion we have the margin of safety study for oral. We have negative DART and genotoxicity studies. We have great dermal and ocular irritation and even dermal sensitization and concentration of use, but we're not evaluating it for air brush.

DR. BERGFELD: Okay.

DR. COHEN: Yeah. And I think for the discussion that the SCC MOS calculation was for oxidized product. We went a little back and forth with safe as used as an oxidative hair dye, but we were comfortable with your conclusion. And we discussed adding another MOS with a more reasonable absorption calculation than the one that we had.

DR. ROSS: That was brought up by PCPC.

DR. BELSITO: Yeah. So, the absorption that I found was 36.3 percent at 16 mgs per kg in DMSO, is that what you want to use for your margin of safety calculation?

DR. ROSS: No. There was a comment on there, we had 0.14 percent at max use. And there was a study which we didn't have 0.14 percent, but we had concentrations which (audio skip) that, which PCPC pointed out, and they wanted to use the 0.5 percent concentration.

DR. COHEN: I'm sorry. Yeah. I misstated it. You're right. That's what it was.

DR. ROSS: And I think the absorption was something like 0.068 percent. So, it's going to be a very comfortable MOS, so I had no problem adding that. And then the other comment was, did we need any description of these hemisulfates in the chemistry? Particularly that was positive on the genotox. I mean, I'm not sure we do but I didn't know if you had a thought on that.

DR. BELSITO: Our team did not discuss that. Curt, Paul, Allan?

DR. ROSS: So, in the chemistry section I'm not even sure we discussed the hemisulfates but --

DR. COHEN: It's in Animal Dermal report.

DR. SNYDER: I certainly didn't see that as a concern.

DR. ROSS: Yeah, it's not a major one, Paul, it's just for completeness really. I'm fine either way.

DR. COHEN: Don, what was the absorption that you mentioned before 38 percent?

DR. BELSITO: It was for a very large amount. I can just delete it (audio skip) .

DR. ROSS: It's for DMSO. I think DMSO changes it.

DR. BELSITO: DMSO 60s mgs per kg.

DR. COHEN: Because otherwise the absorptions very low and the MOS (audio skip) .

DR. BELSITO: Yeah. No, no. This was not in formulation. I mean, I was just looking for the highest level that we had in the report, and questioning if that's what you wanted to use. I don't think it's an appropriate level to use because it is in DMSO. So.

DR. COHEN: No.

DR. ROSS: Yeah. Correct.

DR. KLAASSEN: Yeah, I support calculating the MOS using 0.05 percent. And I think the 50 percent calculation should be removed from the document.

DR. ROSS: Yeah. Curt, we had some discussions around that and whether to take one out or put another one in or whether just the add the one in. And just a question of how conservative did you want to be. And then secondly --

DR. KLAASSEN: You use the data if you have the data. And we have the data. And you know why a lot of times in this risk assessment they use 50 percent, is because you can't be off more than 50 percent. That's true. That's true. As sad as it might sound, that's true. So, we have the data here, and a lot of good data, so use the data.

DR. ROSS: No. I agree with you and that's why I said put that one in. The only reason we left the other one was if we're going with the SCCS methodology, it was if you don't have the exact conditions to use a 50 percent default. And that's why that was in. And I agree totally that if we've got the data, and I agree with the PCPC comments, let's do a more realistic one and let's put in the 0.5 percent absorptions. So that was my sense of it.

DR. KLAASSEN: All right.

DR. BERGFELD: So, the mode of activity here will be that it's going to be voted on as safe and they will, in the background, do this MOS assessment at 0.5? And that will go out with the document once it's approved?

DR. ROSS: Yeah.

DR. BERGFELD: Okay. All right, any other comments before we call for the vote?

DR. RETTIE: With respect to the hemisulfate stuff, which was mentioned earlier, I guess by Dave, do you want to put a structure In there under chemistry just for the hemisulfate, since it's discussed in the text with respect to toxicity?

DR. ROSS: You would do that?

DR. RETTIE: It's a very simple thing to do.

DR. ROSS: Yeah. That'd be great. Thank you.

DR. RETTIE: I like that.

DR. BERGFELD: Anything else? Okay. I'm going to call the question then. And the statement was it was safe as used. All those against? Abstaining? Unanimously accepted then, with the caveat that we're doing the MOS on 0.5 percent. Moving onto other items, Dr. Cohen, pyrogallol.

DECEMBER 2-3, 1998 PANEL MEETING

Dr. Belsito recalled that the following informal data requests on this group of ingredients were issued at the September 10, 1998 Team meeting:

(1) Concentration of use
(2) Physical and chemical properties
(3) Method of manufacture
(4) Impurities data, especially regarding the presence of *m*-cresol
(5) UV absorption data; if absorption occurs in the UVA or UVB range, photosensitization data may be needed
(6) Types of hair dye products (semi-permanent or oxidative) and the rate of reaction (bioavailability)
(7) Metabolism data; if metabolism is not similar to that of 4-Amino-2-Hydroxytoluene and *p*-, *m*-, and *o*-Aminophenol (ingredients already reviewed by CIR), the following data are needed:
    a. 28-day dermal toxicity data with histopathology
    b. dermal reproductive toxicity data
    c. two genotoxicity studies, one using a mammalian system; if positive, a 2-year dermal carcinogenicity study performed using NTP methods.

Dr. Belsito noted that because these ingredients are used in hair dyes and because hair dyes are exempt from sensitization and photosensitization testing as long as the requirement of testing prior to use appears on the label, his Team determined that item 5 above could be deleted. Dr. Belsito said that item 5 should be deleted, because, even if the UV absorption data were positive, the Panel would not have the authority to ask for photosensitization data.

Dr. Schroeter agreed with the revised list of data requests (item 5 deleted).

Dr. Bailey said that he is unsure of how the legal and regulatory status of an ingredient impacts the CIR review process. He said that if there are data that relate to safety, regardless of whether the FDA has legal authority to act, these data should still be of concern to the Panel.

Dr. Belsito said that even if the ingredients were found to be photosensitizers, this would not be a reason for saying that they are unsafe for use in hair dyes, because hair dyes carry a warning about possible sensitization and the need to test prior to use.

Dr. Bailey said that photosensitization is not necessarily being referred to in this case, but, more so, contact sensitization.

Dr. McEwen said that the CIR Procedures do not preclude the Panel from requesting any data that are needed. He said that the Panel needs to determine whether the patch test requirement on the product label sufficiently addresses the Panel's concern about photosensitization, not from a theoretical standpoint, but from the use standpoint of hair dyes.

Concerning the list of data needs included at the beginning of this section, Dr. Belsito said that items 1-4 and 6-7 should be requested for all of the ingredients included in the review. He also reiterated that if the metabolism of these ingredients is not similar, then additional data (e.g. 28-day dermal toxicity data) will be needed.

Dr. McEwen asked if the Panel could use the information on skin penetration from Dr. Walters' presentation to do some modeling on these ingredients to determine if 28-day dermal toxicity data would be needed. In other words, he wanted to know if the Panel would agree to review skin penetration modeling data before requesting 28-day dermal toxicity data.

Dr. Andersen said that after reviewing skin penetration modeling data, the Panel has the option of issuing an Insufficient Data Announcement if these data are not found to be sufficient.

Dr. Belsito said that Dr. Walters presented models that were based on absorption against a barrier of the stratum corneum and data indicating that the forehead is a very absorptive surface, more so than other areas of the body. Dr. Belsito also noted that the follicular shunting mechanism (which is discounted by the models, because, in general, it is not a major area of absorption) would be much more important for a hair dye. Dr. Belsito said that if the skin modeling results indicated a high extent of ingredient absorption, then the 28-day dermal toxicity data would be needed. However, he said that if the results indicated low absorption, he would still want to know what the results would be in a mouse or human, both of which have many hair follicles. He concluded that the computer-generated model would not be useful to him in the present safety assessment.

Someone in the audience commented that the models were generated on specific chemical compounds with similar structures, and that it is possible that the Panel will need absorption data on all four hair dyes included in the safety assessment in order to generate the model.

Dr. Klaassen said that having heard the presentation on skin absorption, he would like for the Panel to include the octanol/water partition coefficient in its request for data on chemical and physical properties. He said that this is the most important chemical parameter that the Panel could have on any ingredient.

Dr. Bailey urged the Panel to be very cautious and be sure to ask certain questions before compounds (especially aromatic amines) are grouped for review in a single report and, potentially, data on one ingredient are wrongfully extrapolated to others.

Dr. Andersen said that the effort by CIR to maximize the benefit from the effort of each review may lead to the creation of as large a family of ingredients as is reasonable. He noted that during reviews by the Panel, any Panel member has an opportunity to recommend the exclusion any ingredient(s) that should not be included in the review.

Dr. Bailey recommended that for ingredients that are reviewed as groups, a table should be created (as part of the report) that indicates which tests have been done on which ingredients.

Dr. Bergfeld said that it was brought to her attention by Dr. Belsito and others that there was a recent hair dye study (4,000 individuals) showing some safety parameters that should be incorporated into CIR's data bank and, perhaps, should be made available for use in the present safety assessment.

Dr. Bailey said that another hair dye study by the American Cancer Society will be published soon. He said that this is a follow-up study to one that was done a few years ago.

Based on the preceding discussion, the following data are needed for completion of the safety assessment on 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 (data needed on all ingredients):

(1) Concentration of use
(2) Physical and chemical properties
(3) Method of manufacture
(4) Impurities data, especially regarding the presence of m-cresol
(5) Types of hair dye products (semi-permanent or oxidative) and the rate of reaction (bioavailability)
(6) Metabolism data; if metabolism is not similar to that of 4-Amino-2-Hydroxytoluene and *p*-, *m*-, and *o*-Aminophenol (ingredients already reviewed by CIR), the following data are needed:
    a. 28-day dermal toxicity data with histopathology
    b. dermal reproductive toxicity data
    c. two genotoxicity studies, one using a mammalian system; if positive, a 2-year dermal carcinogenicity study performed using NTP methods.

Note: The Panel responded to a suggestion that skin penetration modeling might help resolve some of the questions by noting that such an approach probably would not be useful for products that are used on the hair follicle rich scalp and could also contact the skin of the forehead.

An Insufficient Data Announcement containing the preceding data requests will be issued.

JUNE 14-15, 1999 PANEL MEETING

Dr. Belsito recalled that an insufficient data announcement with the following data requests was issued at the December 2-3, 1998 Panel meeting.

(1) Concentration of use
(2) Physical and chemical properties
(3) Method of manufacture
(4) Impurities data, especially regarding the presence of *m*-cresol
(5) Types of hair dye products (semi-permanent or oxidative) and the rate of reaction (bioavailability)
(6) Metabolism data; if metabolism is not similar to that of 4-Amino-2-Hydroxytoluene and *p*-, *m*-, and *o*-Aminophenol (ingredients already reviewed by CIR), the following data are needed:
    a. 28-day dermal toxicity data with histopathology
    b. dermal reproductive toxicity data
    c. two genotoxicity studies, one using a mammalian system; if positive, a 2-year dermal carcinogenicity study performed using NTP methods.

Note: The Panel responded to a suggestion that skin penetration modeling might help resolve some of the questions by noting that such an approach probably would not be useful for products that are used on the hair follicle rich scalp and could also contact the skin of the forehead.

Dr. Belsito noted that, of the data requests listed, current concentration of use data and impurities data (only on 4-amino-*m*-cresol) were received from the cosmetics industry. He also stated that the CIR report contains a good amount of genotoxicity data on some, but not all, of the ingredients and that there is no information indicating how these chemicals are metabolized. Thus, his Team concluded that the current report is insufficient for arriving at a conclusion on the safety of these ingredients in cosmetics.

Dr. Belsito said that if the Panel continues to need data on chemical and physical properties, including the octanol/water partition coefficient, then impurities data (especially, regarding the presence of *m*-cresol and other organic molecules and heavy metals - modification of item 4 above) are needed. He noted that the impurities data are needed on all ingredients except 4-amino-*m*-cresol (data already received on this ingredient). Dr. Belsito added that the Panel still needs items 5 and 6 from the list of data needs, and that item 6c should refer to genotoxicity studies on 5-Amino-4-Chloro-o-Cresol and 5-Amino-6-Chloro-o-Cresol.

Dr. Schroeter said that his Team requested that 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol also be added to item 6c.

Dr. Shank said that a mammalian mutagenicity assay is needed on 4-Chloro-2-Aminophenol and that both mammalian and bacterial mutagenicity assays are needed on 6-Amino-*o*-Cresol.

The Panel voted unanimously in favor of issuing a Tentative Report with an insufficient data conclusion on 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 data needed in order for the Panel to complete its safety assessment of this group of ingredients are listed in the report discussion as follows:

(1) Physical and chemical properties, including the octanol/water partition coefficient
(2) Impurities data, for all except 4-Amino-m-Cresol, especially regarding the presence of heavy metals, *m*-cresol, and other organic molecules
(3) Types of hair dye products (semi-permanent or oxidative) in which these ingredients are used and the rate of reaction (bioavailability) in the hair dye product
(4) Metabolism data; if metabolism is <u>not</u> similar to that of 4-Amino-2-Hydroxytoluene and *p*-, *m*-, and *o*-Aminophenol (ingredients already reviewed by CIR), the following data are needed:

a. 28-day dermal toxicity data with histopathology
b. dermal reproductive and developmental toxicity data
c. for 5-Amino-6-Chloro-*o*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, and 6-Amino-o-Cresol, two genotoxicity studies, one using a mammalian system; for 4-Chloro-2-aminophenol, one genotoxicity study in a mammalian system; if any of these tests for any ingredient are positive, a 2-year dermal carcinogenicity study performed using NTP methods may be needed.

DECEMBER 20-21, 1999 PANEL MEETING

Because a significant amount of data was received one week before the Panel meeting, the Panel voted in favor of tabling any further discussion on this group of ingredients until the February 14-15, 2000 Panel meeting.

FEBRUARY 14-15, 2000 PANEL MEETING

Dr. Belsito noted that the report on this group of ingredients was tabled at the December 20-21, 1999 Panel meeting because of the large data submissions on 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol that were received. He also noted that additional data on 6-Amino-*m*-Cresol and 4-Amino-*m*-Cresol were received prior to today's meeting. Some of the information received indicates that these two dyes could be used in oxidative hair dyes. However, information indicating whether or not they are used in nonoxidative or semipermanent hair dyes was not received.

After reviewing all of the data on the safety of these ingredients, Dr. Belsito's Team concluded that all six are safe as used in oxidative hair dyes and that the following ingredients are safe as used in nonoxidative hair dyes: 6-Amino-*m*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, and 5-Amino-6-Chloro-*o*-Cresol. The Belsito Team also concluded that the available data are insufficient for determining the safety of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol in nonoxidative hair dyes, and that the data needs that were included in the Tentative Report (issued at June 14-15, 1999 Panel meeting) are applicable to these two ingredients.

Dr. Andersen noted that the Belsito Team's conclusion differs significantly from the conclusion that was issued in the Tentative Report (i.e., insufficient data conclusion on all six ingredients). Thus, if the proposed conclusion is approved, the Panel should issue a Revised Tentative Report.

It was moved and seconded that 6-Amino-*m*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, and 5-Amino-6-Chloro-*o*-Cresol are safe as used in oxidative and non-oxidative hair dyes, that 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol are safe as used in oxidative hair dyes, and that the available data are insufficient for supporting the safety of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol in non-oxidative hair dyes. The data that are needed in order for the Panel to complete the safety assessment of these two ingredients are listed in the discussion section of the report as follows:

(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) 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 are needed:
  (a) 28-day dermal toxicity data with histopathology
  (b) dermal reproductive toxicity data
  (c) an *in vitro* genotoxicity study for 6-Amino-o-Cresol, and a genotoxicity study in a mammalian system for 6-Amino-o-Cresol and 4-Chloro-2-Aminophenol (if any of these data are positive, a two-year dermal carcinogenicity study performed using NTP methods may be needed)

The Panel voted unanimously in favor of issuing a Revised Tentative Report with the conclusions stated in the preceding paragraph.

SEPTEMBER 11-12, 2000 PANEL MEETING

Dr. Belsito recalled that at the February 14-15, 2000 Panel meeting, the Panel concluded 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 non-oxidative semipermanent hair dyes, and that the available data also support the safety of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol as used in oxidative hair dyes. The Panel also concluded that the available data are insufficient to support the safety of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol in nonoxidative semipermanent hair dyes. The issuance of a Revised Tentative Report with these conclusions was unanimously approved. Dr. Belsito noted that no data submissions in response to the insufficient data conclusion have been received.

The Panel voted unanimously in favor of issuing a Final Report on this group of ingredients 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 non-oxidative (semi-permanent) 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 non-oxidative (semi-permanent) hair dyes. The data that are needed in order for the Panel to complete its safety assessment of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol are listed in the discussion section of the report as follows:

(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 for all ingredients except 4-Amino-*m*-Cresol
(3) Metabolism data; if 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.

Dr. Belsito recommended that the last paragraph in the report discussion, which includes the data needs stated above, be reworded to clarify that the data needs listed refer to the data that are needed in order for the Panel to assess the safety of 6-Amino-*o*-Cresol and 4-Chloro-2-Aminophenol for use in non-oxidative hair dyes.

Amended Safety Assessment of 4-Amino-*m*-Cresol as Used in Cosmetics

Status:	Draft Final Amended Report for Panel Review
Release Date:	September 6, 2024
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The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume, M.B.A. This safety assessment was prepared by Christina Burnett, M.S., Senior Scientific Analyst/Writer, CIR.

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ABBREVIATIONS

ADME	absorption, distribution, metabolism, and excretion
A_{max}	absorbance maximum
AUC_{last}	area under the curve to last measurable plasma concentration
AUC_{∞}	area under to curve to infinity
C_{max}	maximum observed concentration
CHO	Chinese hamster ovary
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
DMSO	dimethyl sulfoxide
EC_3	estimated concentrations for a SI of 3
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
EU	European Union
FD&C Act	Food, Drug, and Cosmetic Act
FDA	Food and Drug Administration
HPLC	high-performance liquid chromatography
HPLC-UV/RAD/Q-ToF	HPLC coupled to ultraviolet and radiochemical detection/quadrupole time-of flight
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LLNA	local lymph node assay
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MOE	margin of exposure
MOS	margin of safety
NMR	nuclear magnetic resonance
NOAEL	no-observed-adverse-effect-level
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
P_{app}	apparent permeability coefficient
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dose
SI	stimulation index
$t_{1/2}$	half-life
T_{max}	time to C_{max}
TG	test guideline
US	United States
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) reassessed the safety of 4-Amino-*m*-Cresol, which is reported to function as an oxidative hair dye in cosmetic products. The Panel reviewed the available data to determine the safety of this ingredient. The Panel issued an amended report with a revised conclusion stating 4-Amino-*m*-Cresol is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

4-Amino-*m*-Cresol, which according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*) is reported to function in cosmetics as a hair colorant,[1] previously reviewed by the Panel as part of a safety assessment of 6 amino-cresol hair dye ingredients that was published in 2004.[2] At that time, the Panel concluded that "the available data … support the safety of 4-Amino-*m*-Cresol… for use in oxidative and nonoxidative (semi-permanent) hair dyes." 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 several of the other amino-cresol hair dye ingredients that were included in the original 2004 report being banned for use in cosmetics by the European Commission.[3] However, because the Panel determined that data for these amino-cresol hair dye ingredients could not be read-across, rather than including all 6 ingredients in one amended report, re-reviews of each hair dye will be presented as individual stand-alone reports.

Excerpts from the summaries of the previous report on 4-Amino-*m*-Cresol are disseminated throughout the text of this re-review document, as appropriate, and are identified by *italicized text*. (These data are not included in the tables or the Summary.)

Most of the new data included in this safety assessment were found in the opinion of the Scientific Committee on Consumer Products (SCCP)[4] and in the dossier on 4-Amino-*m*-Cresol from the European Chemicals Agency (ECHA).[5] Please note that these sources provide summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when the SCCP and ECHA are cited.

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

CHEMISTRY

Definition and Structure

4-Amino-*m*-Cresol is used as an oxidative hair dye.[4] According to the *Dictionary*, 4-Amino-*m*-Cresol (CAS No. 2835-99-6) is the substituted aromatic compound that conforms to the structure in Figure 1.[1] However, the use of regiochemical terms such as "*meta*-" (i.e., the "-*m*-" in 4-Amino-*m*-Cresol) is vague and inappropriate when an aromatic system such as a benzene ring has more than 2 substituents. Thus, a technical name, such as 4-amino-3-methylphenol, is more common in the literature. Additionally, this ingredient may be supplied as a hemisulfate salt (i.e., 2 equivalents of 4-Amino-*m*-Cresol per 1 equivalent of sulfuric acid; no CAS No.).[4]

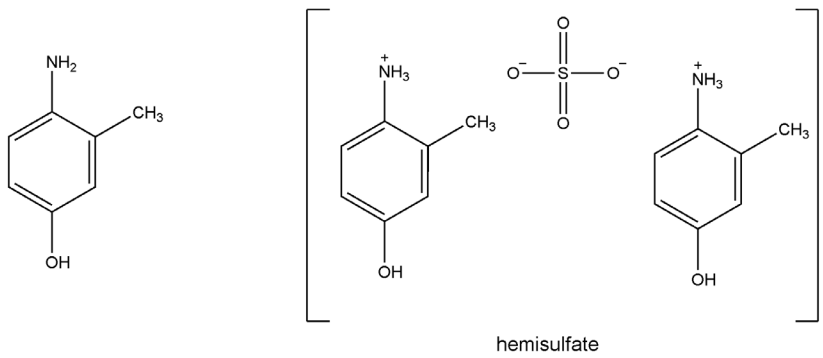

Figure 1. 4-Amino-*m*-Cresol

Chemical Properties

Chemical properties for 4-Amino-*m*-Cresol are summarized in Table 1. 4-Amino-*m*-Cresol (molecular weight 123 g/mol) has a log P_{ow} of 0.51.[6] 4-Amino-*m*-Cresol has a symmetrical absorption peak below 300 nm, with maxima at 206,

234, and 300 in ethanol.[2] The hemisulfate (formula weight of 344.48 g/mol) comprises 2 ammonium cations (i.e. 2 equivalents of "4-ammonium-*m*-cresol") and 1 divalent anion (sulfate), resulting in an estimated log P_{ow} of 0.89.[7]

Method of Manufacture

Method of manufacturing data for 4-Amino-*m*-Cresol were not included in the original report and were not found in the updated literature search, and unpublished data were not submitted. However, non-amino-substituted cresols are traditionally obtained via distillation of coal tar.[8]

Composition and Impurities

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%.[2] 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 high-performance liquid chromatography (HPLC).

The purity of 4-Amino-*m*-Cresol ranged from 94.5 - 99.8% (w/w) through nuclear magnetic resonance (NMR) spectroscopy of 9 different batches and 97.6 - 105.6% % (peak area) through HPLC.[6] Potential impurities of 4-Amino-*m*-Cresol include 3-methyl phenol, sulfanilic acid, and 4-nitro-3-methylphenol, which are raw materials for synthesis of the ingredient, were not detected via analysis.

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 its use in airbrush delivery systems. Data included herein were obtained from 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 were provided by cosmetic product categories, based at that time on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP data, 4-Amino-*m*-Cresol has 28 reported uses in hair dyes and colors.[9] The results of the concentration of use survey conducted by the Council in 2021 (provided in 2022) reported that the maximum concentration of use range of 4-Amino-*m*-Cresol is 0.08 - 0.14% in hair dyes and colors.[10] When the original safety assessment was published in 2004, 4-Amino-*m*-Cresol was reported to have no uses, according to 1998 VCRP data.[2] However, according to industry data from 1999, 4-Amino-*m*-Cresol was reported to be used at up to 0.3% in hair dyes and colors.

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

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

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

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

In the European Union (EU), 4-Amino-*m*-Cresol is categorized in Annex III, the list of substances which cosmetic products must not contain except subject to the restrictions laid down.[13] For this ingredient, the regulation states that the maximum concentration applied to hair or eyelashes must not exceed 1.5% after mixing under oxidative conditions, and dyeing eyelashes is for professional use only. The SCCP concluded that 4-Amino-*m*-Cresol, 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.[6]

TOXICOKINETIC STUDIES

Dermal Absorption

In Vitro

The dermal absorption/percutaneous penetration potential of 4-Amino-*m*-Cresol (95.8% pure) through excised pig skin (840 µm thick) was determined from a commercial hair dye formulation.[6] The study was performed in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 428. The concentration of 4-Amino-*m*-Cresol in the aqueous cream formulation was 1.5%. Using Franz diffusion chambers, 1.5 mg of 4-Amino-*m*-Cresol was applied to the skin (400 mg formulation containing 1.5% 4-Amino-*m*-Cresol applied to 4 cm^2 skin) for 30 min. Application of the test material was terminated by gently rinsing with 4 ml water, then once with a diluted shampoo washing solution, and then twice again with water. The washing solutions were combined, and the amount of 4-Amino-*m*-Cresol was determined by HPLC. The receptor fluid (physiological phosphate buffer containing sodium chloride and antibiotics) was analyzed at 16 and 24 h post-application. At study end, the skin was extracted, and 4-Amino-*m*-Cresol content was quantified.

The penetration rate was 0.7 to 1.1% of the applied dose. The total recovery was 96.1 $\pm$ 3.0% of the applied dose; preliminary stability tests showed that only about 5% of the initial quantity of the applied dose could be recovered after 16 h in the receptor fluid. This indicated a moderate to rapid decrease of 4-Amino-*m*-Cresol when added to the receptor fluid or brought into skin contact. The majority of 4-Amino-*m*-Cresol remained on the skin surface at 95.6 $\pm$ 3.1% of the applied dose. The quantification of the skin penetration by analysis of skin-extracts and the receptor fluid was found not meaningful. 4-Amino-*m*-Cresol was not detected in the receptor fluid. Thus, the limit of detection (equal to 132 ng/cm^2) was used to estimate the content for this compartment, i.e., 0.3 µg/cm^2 (equal to <0.02% of the applied dose). However, due to the instability of the test item in the receptor fluid, this approach was thought not to reflect real absorption conditions. Additionally, the content quantified in the skin as 8.0 ± 2.2 µg/cm^2 (equal to 0.5 ± 0.2 % of the applied dose) might not reflect the real conditions, due to the limited stability of the test item upon skin contact. The amount of 4-Amino-*m*-Cresol bioavailable from a commercial hair dye formulation was determined to range from 8.3 to 66.5 µg/cm^2, which is equivalent to 0.6 to 4.4% of the applied dose. The SCCP determined the study was inadequate based on the large variation in the bioavailability.[6]

In another dermal absorption study, [^{14}C]4-Amino-*m*-Cresol was studied at concentrations of 0.1, 0.5, 1.5, or 2% in formulation with hydrogen peroxide and a reaction partner using excised pig skin.[5] The study was performed in accordance with OECD TG 428. Approximately 400 mg of the test material was applied for 30 min to an exposure area of 4 cm^2. A total of 6 skin samples were used per concentration group for all but the 1.5% group (this group contained 12 samples). At the end of the exposure period, the samples were washed off with water and shampoo and the content of the test material in the rinsing solution was measured. The receptor fluid was sampled at 16, 24, 40, 48, 64, and 72 h intervals to determine the test material concentration. The skin samples were analyzed for radioactivity.

The majority of the test material was recovered in the rinsing solution (95.36, 91.86, 90.98, and 88.6% for the 0.1, 0.5, 1.5, and 2% concentration groups, respectively). Small amounts of radioactivity were detected in the upper skin, lower skin, and receptor fluid for each concentration tested. The total recovery ranged from 92.6% to 103.5%. For all concentrations tested, the material in the receptor fluid was predominantly detected within the first 16 h. The biologically available amounts of radiolabeled 4-Amino-*m*-Cresol were 0.061 $\pm$ 0.011, 0.313 ± 0.112, 0.858 $\pm$ 0.482, and 1.110 $\pm$ 0.611 µg/cm^2 (equivalent to 0.064, 0.068, 0.06, and 0.06%, respectively) for the 0.1, 0.5, 1.5, and 2% concentration groups, respectively.[5]

In a dermal absorption and metabolism study, [^{14}C]4-Amino-*m*-Cresol in a cream formulation mixed with a reaction partner was applied (150 mg) to human skin samples (thickness 0.387 to 0.695 µm) in 2-compartment static diffusion cells.[5] After an application period of 1 h, receptor fluid (Dulbecco's Minimum Eagle Medium and Ham F12 culture medium (3:1)) samples were collected at 3 h and 24 h to determine the amount of parent compound as well as the metabolic profile. The mean absorption of the test material was 0.26 $\pm$ 0.09% of dose applied (3.89 $\pm$ 1.37 µg/cm^2). Total recovery of the applied dose was 99.5 $\pm$ 1.7%. 4-Amino-*m*-Cresol was almost completely metabolized or converted, with 4-(acetylamino)-3-methyl-phenol seeming to account for a major portion of the profiled radioactivity.

Animal

Dermal absorption of [^{14}C]4-Amino-*m*-Cresol (hemisulfate) was studied in groups of 3 male and 3 female pigmented PVG rats.[6] The test substance was applied to an area of 9 cm^2 at concentrations of 15 % in dimethyl sulfoxide (DMSO) and of 1.5 % in a commercial formulation with hydrogen peroxide, for 24 h and 30 min contact, respectively. Mean dose applied was 1.611 mg/cm^2 for the material in DMSO and 1.516 mg/cm^2 for the commercial formulation. The treated skin was covered with an occlusive plaster during the exposure period. After the exposure period ended, the test substance was scraped off (formulation only) and the skin was rinsed with a shampoo formulation and warm water. After rinsing, the area was covered with an aluminum foil strip and securely sealed by an occlusive plaster to further prevent licking of the treated area during the 72 h in the metabolism cages. Details on systemic distribution and excretion are described in the following section under dermal animal studies.

Total recovery of the applied radioactivity was 86.7 and 89.8% for males and females, respectively, treated with the commercial formulation and 95.7 and 97.9 % for males and females, respectively, treated with the DMSO solution. The amount of radioactivity remaining on the skin for the cosmetic formulation represented 2.8 and 1.73% of the applied dose for males and females, respectively. For the DMSO solution, the radioactivity remaining on the skin was 7.86 and 6.1% of the applied dose for males and females, respectively. The majority of the applied doses were recovered in the dressings and the washing solutions, representing 83.3 - 87.7% and 74.4 - 81.6% of the applied amount for the formulation and the DMSO solution, respectively. A cutaneous absorption of 2.73% of applied dose (equivalent to 41.4 ug/cm²) was determined for a commercial formulation applied under typical use conditions in the presence of peroxide. The respective amount for the DMSO solution is 14.38 % of the applied dose (equivalent to 231.7 µg/cm²). No significant differences were noted between males and females with regard to skin absorption of 4-Amino-*m*-Cresol when applied in either a hair dye formulation or a DMSO solution.[6]

Absorption, Distribution, Metabolism, and Excretion (ADME) Studies

In Vitro

The metabolic profile of 4-Amino-*m*-Cresol was investigated in vitro using primary hepatocytes from male human donors, male Sprague Dawley rats, and male ICR/CD-1 mice.[6] The metabolic capacity of the hepatocytes was characterized by marker substrates for phase I (general cytochrome P450 activity for humans and rodents and specific activity of 1A1/2 and 2E1) and phase II enzymes (*N*-acetyl-transferase 1/2). Approximately 1 x 10^6 cells/ml were incubated with 10 µM (mouse hepatocytes) or 6.6 µM (rat, human hepatocytes) of 4-Amino-*m*-Cresol for 4 h. Samples of the supernatant were taken and analyzed at 0, 0.5, 1.5, and 4 h. The metabolic stability was assessed by detection of loss of parent compound by means of liquid chromatography with tandem mass spectrometry (LC-MS/MS). The metabolic profile was also studied using LC-MS and metabolites identified/characterized as far as possible. Cell viability (90%, 95% and 80% in human, rat, and mouse, respectively) was not affected by 4-Amino-*m*-Cresol during the incubation period. A slight decrease in viability of about 10% was noted for the end of the entire incubation period. The marker enzymes demonstrated the metabolic capacity and the validity of the test system. Differences in the metabolic capacity between rat, mouse and human hepatocytes were observed for the different phase I marker reactions. 4-Amino-*m*-Cresol revealed a rapid rate of metabolism in rat and human hepatocytes. A decrease of 95.2 and 89.8% of the parent compound was detected within 1.5 h incubation for human and rat hepatocytes, respectively. The mouse incubation could not be analyzed due to analytical problems. The analysis of the formed metabolites revealed an intensive phase II metabolism resulting in sulfation of the phenol group for all three species. In contrast, no indication of *N*-acetylation was noted for rat or human hepatocytes. The findings from studies on rat and human hepatocytes, under the same experimental conditions, show no significant difference in metabolic rate/capacity or metabolic profiles between these cells. The authors stated the outcomes of this comparative in vitro metabolism investigation in hepatocytes support the applicability of extrapolating from rat data to humans in terms of liver metabolism.

A similar metabolic profile study was performed using human cryopreserved hepatocytes that were either suspended or plated prior to exposure to 4-Amino-m-Cresol.[5] The concentrations tested in the suspended hepatocytes was 1 or 10 µg/ml 4-Amino-*m*-Cresol, and in the plated hepatocytes, the concentrations tested were 0.862, 8.62, or 24.6 µg/ml 4-Amino-*m*-Cresol. Testing was performed in triplicate. The suspended cells were incubated for 3 h and the plated cells were incubated for 24 h. The formation of the metabolites was analyzed by high-performance liquid chromatography-coupled to ultraviolet and radiochemical detection/quadrupole-time-of flight (HPLC-UV/RAD/Q-ToF)-mass spectrometry. In the suspended hepatocytes, a glucuronide metabolite of 4-Amino-*m*-Cresol was formed, but it could not be determined if it was conjugated to *N* or *O*. A 10-fold increase in the test material dose did not result in a 10-fold increase in the glucuronide metabolite production, indicating the enzyme might have become saturated. In the plated hepatocytes, *N*-acetyl-4-amino-*m*-cresol and glucuronide metabolites of 4-Amino-*m*-Cresol were observed. Lower levels of metabolites were produced at the high concentration compared to the metabolites produced at the lower 2 concentrations, which may have been due to adverse effects on the hepatocytes at the highest concentration tested. The positive control yielded expected results.

In another similar study using immortalized human keratinocyte HaCaT cells, 4-Amino-*m*-Cresol was tested at 0.862, 8.62, and 24.6 µg/ml.[5] The cells were incubated after dosing for 3 or 24 h. One metabolite, *N*-acetyl-4-amino-*m*-cresol, was observed in the incubations at both 3 and 24 h intervals in all test concentrations.

In an in vitro study, the bioavailability of 4-Amino-*m*-Cresol across the intestinal barrier was investigated in human intestinal epithelial (TC-7) cells.[4] The permeability from the apical to the basolateral side was studied in 96- transwell plates with shaking for a 60 min contact time. Analysis of the donor (apical) and receiver (basolateral) samples was done by means of HPLC-MS/MS and the apparent permeability coefficient (P_{app}) was calculated for 2 independent experiments. Approximately 4 µM [^{14}C]mannitol was used to demonstrate the integrity of the cell monolayer. Only monolayers revealing a permeability of < 2.5 x 10^{-6} cm/sec were used. The validity of the test system was demonstrated by analyzing propranolol, vinblastine, and ranitidine. According to the laboratory's classification system, a low permeability is considered for test items revealing a P_{app} < 2 x 10^{-6} cm/sec. A P_{app} of 2 - 20 x 10^{-6} cm/sec and a $P_{app} \geq 20 \times 10^{-6}$ cm/sec classify a substance to have a moderate and a high permeability, respectively. Ranitidine was used as a low permeability reference compound (it has a 50 % absorption in humans with a P_{app} of 0.4 x 10^{-6} cm/sec in the test). The total recovery for the reference substances and 4-Amino-*m*-Cresol ranged from 83 to 100%. A P_{app} of 59 x 10^{-6} cm/sec was determined for 4-Amino-*m*-Cresol, and thus was classified to be of high permeability, indicating nearly 100% absorption from the gastrointestinal tract.

Animal

Dermal

The dermal absorption study of [^{14}C]4-Amino-*m*-Cresol (hemisulfate) described in the above section also studied the excretion of the test material in the groups of 3 male and 3 female pigmented PVG rats.[6] The test substance was applied to an area of 9 cm^2 at concentrations of 15 % in DMSO and of 1.5 % in a commercial formulation with hydrogen peroxide, for 24 h and 30 min contact, respectively. Mean dose applied was 1.611 mg/cm^2 for the material in DMSO and 1.516 mg/cm^2 for the commercial formulation. Urine and feces were collected daily (0 - 24, 24 - 48, and 48 - 72 h after administration) from the cages. Exhaled carbon dioxide was removed every 24 h for the 72-h post-dosing period. Animals were killed 72 h after application, and the application sites, blood, and organs were analyzed for radioactivity. The radioactivity in the remaining carcass was also determined.

Total recovery of the applied radioactivity was 86.7 and 89.8% and 95.7 and 97.9% for males and females treated with the commercial formulation and the DMSO solution, respectively. Absorbed radioactivity was mainly excreted via urine both for the commercial formulation (0.35% males, 0.15% females) and for the DMSO solution (8.09% males, 5.00% females), with 79.4 - 88.9% of the total amount being excreted within the first 24 h. Excretion via feces was 0.01 - 0.02% for the formulation and 0.27 - 0.58% for the DMSO solution. Elimination via expiration was less than 0.2% for both the commercial formulation and the DMSO solution. Low levels of radioactivity were detected in all tissues examined for the DMSO solution, except for the thyroid, adrenal and gonads, in which detectable amounts were only found in one sex and/or for some but not all of the animals within one group. The highest levels were noted for the remaining carcass (0.059 - 0.063% of the applied dose) and the gastrointestinal tract content (0.006 - 0.007% of the applied dose). Similar findings were noted for the formulation, but the tissue levels were in general lower than the ones noted for the DMSO solution. Again, the highest levels were noted for the remaining carcass and the gastrointestinal-tract content as observed with the DMSO solution. No significant differences were noted between males and females with regard to tissue-distribution and elimination of 4-Amino-*m*-Cresol when applied with in either a hair dye formulation or a DMSO solution.[6]

The ADME and toxicokinetics of 4-Amino-*m*-Cresol was studied in female Wistar rats.[5] Groups of 4 - 6 rats received 12 or 60 mg/kg bw radiolabeled 4-Amino-*m*-Cresol (0.2 ml) either in cream formulation or DMSO applied uniformly onto shaved skin. The study was performed in accordance with OECD TG 417 and TG 427. O-rings were utilized in the application of the test material and the total dosing surface area was 20 cm^2 per animal. Animals wore a collar to prevent disruption of the treatment area. Exposure duration was 0.5 h in the low dose group and 24 h in the high dose group. The test substance was washed from the skin at the end of the exposure period and the application sites were tape-stripped. Animals were observed for mortality, clinical signs, and body weight before they were killed. Urine and feces were collected from the ADME group pre-dosing and during 4 intervals over 72 h. Cage rinses, wash, O-rings, and tape strips were collected at termination. Blood and tissue samples for the ADME group were collected after the animals were killed. Blood samples from the toxicokinetics group were collected at 0.25, 0.5, 0.75, 1, 2, 4, 8, 24, 48, and 72 h after dosing. Total percent radioactivity recovered was determined in the ADME group. For the toxicokinetic group, maximum observed concentration (C_{max}), time to C_{max} (T_{max}), half-life ($t_{1/2}$), area under the curve to last measurable plasma concentration (AUC_{last}), area under the curve to infinity (AUC_{∞}), and elimination rate were determined from plasma kinetics.

Body weights of all animals were within 20% of the sex mean. No unscheduled deaths were observed. All rats displayed red discharge from the nose and eye after dosing, which was related to wearing the collar. On average, 86 and 54% of the applied radioactivity was recovered from back-wash in the ADME low and high dose group, respectively. The average dermal absorption was 1.05% (or 0.001 mg/cm^2) in the low-dose group (cream formulation after 30 min) and 36.3%, (or 0.28 mg/cm^2) in high-dose group (DMSO after 24 h). In the high-dose group, the average total remaining radioactivity in blood, carcass, and tissues (excluding treated skin) was 0.98% of the administered dose, indicating no major accumulation of test substance after 72 h. The radioactivity detected in treated skin was 2.11% of the administered dose. In the low-dose group, radioactivity was below the detection limit in most tissues, and 0.4% of the administered radioactivity was excreted in urine, indicating poor dermal absorption. In the high-dose group, urinary excretion accounted for 32% of the administered dose, indicating urine was an important route of elimination for the test substance. The highest rate of excretion was observed during the first 8 h and decreasing excretion rate was noted thereafter. The fecal excretion of radioactivity was 0.2% after low dermal dosing and 2.4% after high dermal dosing. No toxicokinetic evaluation was performed for the low dose group, since most plasma data were below the limit of quantification. The toxicokinetic parameters determined from the high-dose toxicokinetics group were as follows: T_{max} value of 2 h, C_{max} was 15.7 mg/kg, AUC_{last} was 81.3 h x mg/kg, AUC_{∞} was 87.4 h x mg/kg, and $t_{1/2}$ was 30.1 h. The elimination rate was determined to be 0.023/h.[5]

Oral

The ADME and toxicokinetics of 4-Amino-*m*-Cresol following oral dosing was studied in female Wistar rats.[5] Groups of 4 - 6 rats received a single oral dose of 60 mg/kg bw radiolabeled 4-Amino-*m*-Cresol via gavage. The study was performed in accordance with OECD TG 417. The vehicle for the test material was 0.5% w/w carboxymethylcellulose and 0.5% w/w ascorbic acid in 0.05 M phosphate buffer. Animals were observed for mortality, clinical signs, and body weight before they were killed. Urine and feces were collected from the ADME group pre-dosing and during 4 intervals over 72 h. Cage rinses were collected at termination. Blood and tissue samples for the ADME group were collected after the animals were killed. Blood samples from the toxicokinetics group were collected at 0.25, 0.5, 0.75, 1, 2, 4, 8, 24, 48, and 72 h after

dosing. Total percent radioactivity recovered was determined in the ADME group. For the toxicokinetic group, T_{max}, C_{max}, $t_{1/2}$, AUC_{last}, AUC_{∞}, and elimination rate were determined from plasma kinetics.

Body weights of all animals were within 20% of the sex mean. No unscheduled deaths were observed. All rats displayed piloerection and brown discoloration of urine after dosing. Excretion in the urine accounted for 92% of the administered dose, with the highest rate of excretion observed during the first 8 h and decreasing excretion rate noted thereafter. Excretion in the feces was 3.9% of the administered dose and was delayed compared to urine, with the highest excretion between 8 and 24 h. Approximately 2.1% radioactivity was recovered in cage wash. The oral absorption, calculated as fractional absorption from the urine data, was 105%. The absolute oral bioavailability calculated from the plasma data was 86%. No major accumulation of radioactivity was observed at 72 h, with the average total radioactivity in blood, carcass, and tissues as 0.82% of administered dose. The highest residual concentration was observed in the liver. From the plasma data, with T_{max} value of 0.25 h showed that oral absorption was fast. C_{max} was 89.1 mg/kg, AUC_{last} was 163 h x mg/kg, AUC_{∞} was 167 h x mg/kg, and $t_{1/2}$ was 24 h. The elimination rate was determined to be 0.0289/h.[5]

Other Route

The same research study that studied the dermal and oral ADME and toxicokinetics of 4-Amino-*m*-Cresol in female Wistar rats described above also investigated an intravenous administration of 60 mg/kg bw of the test material.[5] Groups of 4 - 6 animals were injected once with 12 mg/ml test substance in 0.5% w/w carboxymethylcellulose and 0.5% w/w ascorbic acid in 0.05 M phosphate buffer. The remaining methodology is the same as described above for the oral study.

Body weights of all animals were within 20% of the sex mean. No unscheduled deaths were observed. All rats displayed piloerection and brown discoloration of urine after dosing. Excretion in the urine accounted for 88% of the administered dose, with the highest rate of excretion observed during the first 8 h and decreasing excretion rate noted thereafter. Excretion in the feces was 2% of the administered dose and was delayed compared to urine, with the highest excretion between 8 and 24 h. Approximately 2.6% radioactivity was recovered in cage wash. No major accumulation of radioactivity was observed at 72 h, with the average total radioactivity in blood, carcass, and tissues as 1% of administered dose. The highest residual concentration was observed in the liver. From the plasma data, the T_{max} value of 0.25 h showed that intravenous absorption was fast. C_{max} was 89.1 mg/kg, AUC_{last} was 3.48 h x mg/kg, AUC_{∞} was 197 h x mg/kg, and $t_{1/2}$ was 19 h. The elimination rate was determined to be 0.0364/h.[5]

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

In an acute toxicity study, groups of 6 female CF-1 mice and groups of 5 male and 5 female Wistar rats received single oral doses of 10% 4-Amino-*m*-Cresol in gum arabica via gavage.[6] The doses for the female mice were 750, 800, 850, 900, 950, or 1000 mg/kg bw. The male rats received 700, 800, 900, 1000, or 1100 mg/kg bw, while the female rats received 800, 900, 1000, 1100, or 1200 mg/kg bw. Mortality and clinical signs of toxicity were checked daily for 14 d. Body weights were recorded, and all animals underwent gross necropsy at the end of the observation period. Reduction of physical activity was noted in the animals. Deaths occurred within 2 to 48 h of dosing (number per group not reported). At necropsy, no macroscopic organ changes were observed. The LD_{50} for 4-Amino-*m*-Cresol in the female mice was 908 mg/kg bw. The LD_{50} in female rats was 1010 mg/kg bw, and in male rats, the LD_{50} was 870 mg/kg bw.

Short-Term Toxicity Studies

In a short-term oral toxicity study, groups of 6 male CD-1 mice received 1000, 1200, 1440, 1728, or 2074 mg/kg 4-Amino-m-Cresol (method of oral administration not specified) on 2 consecutive days.[2] The LD_{50} was determined as 1000 mg/kg. At least 1 mouse in all groups survived until day 14 of the observation period, but most mice died on day 1 or 2. Clinical observations included piloerection in all groups, hypokinesia in all but the low-dose group, ataxia in the 1440- and 2074-mg/kg dose groups, and prostration in the 1200 mg/kg dose group.

Subchronic Toxicity Studies

Oral

In a 13-wk oral study, male and female Wistar rats received 4-Amino-m-Cresol by gavage at doses of 0, 15, 60, or 120 mg/kg/d.[2] No clinical observations or pathological findings indicative of systemic toxicity were observed in the 15 mg/kg dose group. The 60 and 120 mg/kg dose groups had dark, discolored urine from weeks 8 to 13 that were attributed to the test material. The 120 mg/kg dose group had significantly increased creatinine values in female rats after 13 wk, although values were still within the normal range. Absolute spleen weights were increased in a statistically significant manner in female rats in the 120 mg/kg dose group (absolute spleen weights were also increased in males, but not in a statistically significant manner). No microscopic changes attributed to the test material were observed. The no-observed-adverse-effect-level (NOAEL) was 60 mg/kg/d.

Repeated-dose toxicity studies of 4-Amino-*m*-Cresol were not found in the updated literature search, and unpublished data were not submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

Groups of 24 female rats (strain BOR:WISW-SPF TNO) were dose orally with 0, 10, 40, or 80 mg/kg 4-Amino-m-Cresol from days 5 to 15 of gestation.[2] Dams were killed on day 20 of gestation. No clinical signs of toxicity nor mortalities were observed in the dams. No abnormalities were observed at gross necropsy. No abnormalities were observed in litter parameters. No malformations were observed in external or skeletal examination of fetuses. Hydrocephaly was observed in 1 fetus of the 40 mg/kg dose group and minor visceral anomalies (increased renal pelvic cavitation) was observed in 2 fetuses in the 80 mg/kg dose group. The malformation index for all groups was 0, except the 40 mg/kg dose group, which had a malformation index of 0.56%. The NOAEL was established at 80 mg/kg.

Development and reproductive toxicity studies for 4-Amino-*m*-Cresol were not found in the updated literature search, and unpublished data were not submitted.

GENOTOXICITY STUDIES

4-Amino-m-Cresol was not mutagenic with or without metabolic activation in an Ames test evaluating concentrations of up to 600 µg/plate, alone and with equal amounts of 6% hydrogen peroxide.[2] 4-Amino-m-Cresol in DMSO did not induce unscheduled DNA synthesis in in vitro male rat primary hepatocytes at up to 100 µg/ml. In in vivo micronucleus tests in mice, 4-Amino-m-Cresol in DMSO or carboxymethylcellulose did not induce micronuclei after a single oral dose of up to 1000 mg/kg bw. In Chinese hamsters, 4-Amino-m-Cresol (hemisulfate) in double distilled water did not cause sister chromatid exchanges at oral doses up to 2000 mg/kg or intraperitoneal doses up to 400 mg/kg. No unscheduled DNA synthesis was induced in rats that received up to 1000 mg/kg 4-Amino-m-Cresol.

Additional in vitro and in vivo genotoxicity studies on 4-Amino-*m*-Cresol summarized here are detailed in Table 2. In Ames tests, 4-Amino-*m*-Cresol was not mutagenic, with or without metabolic activation, at up to 5000 µg/plate.[4,14] 4-Amino-*m*-Cresol (free base and hemisulfate) was not genotoxic in a L5178Y mouse lymphoma cell assays at the *tk* locus at up to 6.25 µg/ml without metabolic activation or at up to 1000 µg/ml with metabolic activation. Clastogenic effects were observed in chromosome aberration tests with 4-Amino-*m*-Cresol (free base and hemisulfate) in human peripheral lymphocytes (tested at up to 100 µg/ml without metabolic activation and at up to 156.25 µg/ml with metabolic activation) and in Chinese hamster ovary (CHO) cells (tested at up to 500 µg/ml without metabolic activation and at up to 2000 µg/ml with metabolic activation).[5] Ambiguous results for genotoxicity were observed in a micronucleus test using human peripheral lymphocytes exposed to up to 150 µg/ml 4-Amino-*m*-Cresol with metabolic activation; without metabolic activation, the test material was not genotoxic. Ambiguous results for genotoxicity were also observed in an unscheduled DNA synthesis assay in rat primary hepatocytes exposed to up to 10.0 µg/ml 4-Amino-*m*-Cresol. In HeLa cells, genotoxicity was observed in an unscheduled DNA synthesis assay of 4-Amino-*m*-Cresol (hemisulfate) at up to 500 µg/ml.

An in vivo micronucleus test found that 4-Amino-*m*-Cresol did not induce micronuclei in the bone marrow cells of mice that received a single intraperitoneal dose of up to 200 mg/kg bw.[6] Unscheduled DNA synthesis was not observed in the hepatocytes of male rats that received a single oral dose of up to 2000 mg/kg bw 4-Amino-*m*-Cresol,[4] however, genotoxicity was observed in the same type of assay performed with up to 600 mg/kg 4-Amino-*m*-Cresol (hemisulfate).[5]

CARCINOGENICITY STUDIES

Carcinogenicity data for 4-Amino-*m*-Cresol were not included in the original report and were not found in the updated literature search, and unpublished data were not submitted.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Dermal irritation and sensitization data on 4-Amino-*m*-Cresol are summarized in Table 3. Undiluted 4-Amino-*m*-Cresol was classified as a non-irritant and non-corrosive in EpiSkin™ and EpiDerm™ assays using human epidermal keratinocytes.[5] A 3% aqueous solution of 4-Amino-*m*-Cresol in 0.5% tylose was not irritating in a study with guinea pigs.[6] In a guinea pig maximization test, 4-Amino-*m*-Cresol was non-sensitizing when induced and challenged at up to 3%; however, the SCCP noted the test design of this study was inadequate and had reporting deficiencies. 4-Amino-*m*-Cresol was a moderate sensitizer in a local lymph node assay (LLNA) when tested at up to 10% in DMSO or up to 5.0% in water/acetone (1:1) mixed with olive oil (4:1). The estimated concentrations for a stimulation index (SI) of 3 (EC_3) were calculated to be 1.45% for the DMSO group and 2.15% for the water/acetone/oil group.

OCULAR IRRITATION STUDIES

In vitro and animal ocular irritation data on 4-Amino-*m*-Cresol are summarized in Table 4. Undiluted and 1.5% 4-Amino-*m*-Cresol in 50% propylene glycol were classified as not irritating in isolated chicken eye tests.[5] In a human cornea model test, undiluted 4-Amino-*m*-Cresol led to a viability of 93.8%; however, the test system was determined not suitable for assessment of the test material because the material was proved to be a strong reducer of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent. In an ocular irritation study using guinea pigs, 1.5% 4-Amino-*m*-Cresol in 50% propylene glycol was determined to have minimal ocular irritant potential.[6]

MARGIN OF EXPOSURE

MOE is a quantitative factor calculated by dividing the NOAEL obtained for an ingredient in an animal experiment by the estimated systemic exposure dose (SED) for the ingredient in humans, generally according to US Environmental Protection Agency (EPA) and European Commission Scientific Committee on Consumer Safety (SCCS) guidelines. The standard MOE value of 100 is derived from multiplying two factors: a 10-fold factor accounts for the extrapolating data from test animals to human being (interspecies extrapolation) and an additional 10-fold for accommodating differences among the human population (intra-species extrapolation). An MOE value greater than 100 has traditionally been considered an indication of safety. The MOE is sometimes referred to as margin of safety (MOS), despite the parameters being definitionally different.

The SCCP calculated an MOE value for 1.5% 4-Amino-*m*-Cresol in a formulation with hydrogen peroxide (on head) to be 124.[6] This calculation is based on the NOAEL of 60 mg/kg bw/d from a 90-d oral rat study and a systemic exposure dose (SED) of 0.483 mg/kg bw (skin area surface of 700 cm^2 x absorption through skin of 41.4 $\mu g/cm^2$ x 0.001 (unit conversion)/ typical human bw of 60 kg; the dermal absorption rate of 41.4 $\mu g/cm^2$ equivalents to 2.73 % of applied dose.).

The 2021 survey provided by the Council in 2022 indicates that the highest usage concentration range for 4-Amino-*m*-Cresol in hair dye products is between 0.08 and 0.14%.[10] In a dermal penetration study using pig skin, bioavailability of 4-Amino-*m*-Cresol at a concentration of 0.5% in formulation with hydrogen peroxide was 0.425 $\mu g/cm^2$ (0.313 $\pm$0.112 $\mu g/cm^2$, mean $\pm$ SD) (with a range of 0.072 to 1.721$\mu g/cm^2$, corresponding to 0.1 to 2% of the dosage applied).[14,15] This yielded an SED value of 0.005 mg/kg bw/d (skin area surface of 700 cm^2 x absorption through skin of 0.425 $\mu g/cm^2$ x 0.001 (unit conversion)/typical human bw of 60 kg). The SED value used herein is significantly smaller compared to the one in the SCCP calculation due to two factors: i) the maximum use concentration of 4-Amino-*m*-Cresol was reduced from 1.5% to 0.14%, and ii) dermal absorption data is now available for a concentration of 0.5% in the formulation, which corresponds to 0.425 $\mu g/cm^2$ (or 0.068% of applied dose). By using an NOAEL of 60 mg/kg bw/d derived from a 13-wk oral study,[2] the Panel calculated the MOE to be 12,000.

$$\text{MOE} = \frac{\text{NOAEL}}{\text{SED}} = \frac{60 \text{ mg/kg bw/d}}{0.005 \text{ mg/kg bw/d}} = 12{,}000$$

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

SUMMARY

4-Amino-*m*-Cresol is reported to function in cosmetics as a hair colorant. 4-Amino-*m*-Cresol was previously reviewed by the Panel as part of a safety assessment of 6 amino-cresol hair dye ingredients that was published in 2004. At that time, the Panel concluded that according to the available data (in that report), 4-Amino-*m*-Cresol is safe for use in oxidative and non-oxidative hair dyes. 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 several of the other amino-cresol hair dye ingredients that were included in the original 2004 assessment being banned for use in cosmetics by the European Commission.

According to 2023 VCRP survey data, 4-Amino-*m*-Cresol has 28 reported uses in hair dyes and colors. The results of the concentration of use survey conducted by the Council in 2021 (provided in 2022) reported that the maximum concentration of use range for 4-Amino-*m*-Cresol is 0.08 - 0.14% in hair dyes and colors. When the original safety assessment was published in 2004, 4-Amino-*m*-Cresol was reported to have no uses, according to 1998 VCRP data. However, according to industry data from 1999, 4-Amino-*m*-Cresol was reported to be used at up to 0.3% in hair dyes and colors.

According to the EU, 4-Amino-*m*-Cresol is categorized in Annex III, the list of substances which cosmetic products must not contain except subject to the restrictions laid down. For this ingredient, the regulation states that the maximum concentration applied to hair or eyelashes must not exceed 1.5% after mixing under oxidative conditions, and dyeing eyelashes is for professional use only. The SCCP concluded that 4-Amino-*m*-Cresol, 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.

In one in vitro dermal penetration study in excised pig skin, the bioavailability of 4-Amino-*m*-Cresol in a commercial hair dye formulation ranged from 8.3 to 66.5 µg/cm^2 (0.6 to 4.4% of the applied dose); however, the SCCP determined the study was inadequate based on the large variation of the bioavailability. In another in vitro study pig skin study of 4-Amino-*m*-Cresol, the ingredient was tested in formulation with hydrogen peroxide and a reaction partner at concentrations of 0.1, 0.5, 1.5, and 2%. The biologically-available amounts of 4-Amino-*m*-Cresol were 0.061 ± 0.011, 0.313 ± 0.112, 0.858 ± 0.482, and 1.110 ± 0.611 µg/cm^2 (equivalent to 0.064, 0.068, 0.06, and 0.06%, respectively) for the 0.1, 0.5, 1.5, and 2% concentration groups, respectively. In a dermal absorption and metabolism study using human skin samples, 4-Amino-*m*-Cresol in a cream formulation mixed with a reaction partner was completely metabolized and the mean absorption was 3.89 ± 1.37 µg/cm^2. The cutaneous absorption of 4-Amino-*m*-Cresol (hemisulfate) in rats was determined to be 41.4 ug/cm² (2.73% of applied dose) for a commercial formulation applied under typical use conditions in the presence of peroxide and 221.7 µg/cm^2 (14.38% of applied dose) for a DMSO solution.

In vitro metabolism studies found that 4-Amino-*m*-Cresol was rapidly metabolized in rat and human hepatocytes and yielded *N*-acetyl-4-amino-*m*-cresol and glucuronide metabolites. An in vitro absorption study using human intestinal epithelial cell line TC-7 cells indicated a good absorption of 4-Amino-*m*-Cresol after oral administration. In a rat dermal study of [^{14}C]4-Amino-*m*-Cresol (hemisulfate) at concentrations of 15 % in DMSO and of 1.5 % in a commercial formulation with hydrogen peroxide, small amount of absorbed radioactivity was mainly excreted in urine, with 79.4-88.9% of the total amount being excreted within the first 24 h. Excretion via feces and respiration was at much lower percentages and little radioactivity was measured in the tissues. With dermal dosing of 12 or 60 mg/kg bw 4-Amino-*m*-Cresol in female rats, the majority of the applied dose was rinsed off. In the low dose group, 0.4% of administered radioactivity excreted in urine, indicating poor dermal absorption. In the high dose group, urinary excretion accounted for 32% of the administered dose, indicating urine as an important route of elimination for test substance. The highest rate of excretion was observed during the first 8 h and decreasing excretion rate noted thereafter. After oral administration to female Wistar Crl:WI rats, 4-Amino-*m*-Cresol was extensively absorbed (105% of the administered dose based on urine data and 86% based on plasma data), extensively metabolized and excreted mainly via the urine (92%). No major accumulation of the test material seems to occur in the body 72 h after administration (0.82%). Intravenous administration of 4-Amino-*m*-Cresol to female rats showed that the majority of the administered dose was excreted in the urine within the first 8 h of dosing.

The LD_{50} for 4-Amino-*m*-Cresol (10%) in the female mice was 908 mg/kg bw. The LD_{50} for the same test material in female rats was 1010 mg/kg bw, and in male rats, the LD_{50} was 870 mg/kg bw.

In Ames tests, 4-Amino-*m*-Cresol was not mutagenic, with or without metabolic activation, at up to 5000 µg/plate. 4-Amino-*m*-Cresol (free base and hemisulfate) was not genotoxic in a L5178Y mouse lymphoma cell assays at the *tk* locus at up to 6.25 µg/ml without metabolic activation or with up to 1000 µg/ml with metabolic activation. Clastogenic effects were observed in chromosome aberration tests with 4-Amino-*m*-Cresol (free base and hemisulfate) in human peripheral lymphocytes (tested at up to 100 µg/ml without metabolic activation and at up to 156.25 µg/ml with metabolic activation) and in CHO cells (tested at up to 500 µg/ml without metabolic activation and at up to 2000 µg/ml with metabolic activation). Ambiguous results for genotoxicity were observed in a micronucleus test using human peripheral lymphocytes exposed to up to 150 µg/ml 4-Amino-*m*-Cresol with metabolic activation; without metabolic activation, the test material was not genotoxic. Ambiguous results for genotoxicity were also observed in an unscheduled DNA synthesis assay in rat primary hepatocytes exposed to up to 10.0 µg/ml 4-Amino-*m*-Cresol. In HeLa cells, genotoxicity was observed in an unscheduled DNA synthesis assay of 4-Amino-*m*-Cresol (hemisulfate) at up to 500 µg/ml.

An in vivo micronucleus test found that 4-Amino-*m*-Cresol did not induce micronuclei in mice that received a single intraperitoneal dose of up to 200 mg/kg bw. Unscheduled DNA synthesis was not observed in the hepatocytes of male rats that received a single oral dose of up to 2000 mg/kg bw 4-Amino-*m*-Cresol; however, genotoxicity was observed in the same type of assay performed with up to 600 mg/kg 4-Amino-*m*-Cresol (hemisulfate).

Undiluted 4-Amino-*m*-Cresol was classified as a non-irritant and non-corrosive in EpiSkin™ and EpiDerm™ assays using human epidermal keratinocytes. A 3% aqueous solution of 4-Amino-*m*-Cresol in 0.5% tylose was not irritating in a study with guinea pigs. In a guinea pig maximization test, 4-Amino-*m*-Cresol was non-sensitizing when induced and challenged at up to 3%; however, the SCCS noted the test design of this study was inadequate and had reporting deficiencies. 4-Amino-*m*-Cresol was a moderate sensitizer in an LLNA when tested at up to 10% in DMSO or up to 5.0% in water/acetone (1:1) mixed with olive oil (4:1). The EC_3 were calculated to be 1.45% for the DMSO group and 2.15% for the water/acetone/oil group.

Undiluted and 1.5% 4-Amino-*m*-Cresol in 50% propylene glycol were classified as not irritating in isolated chicken eye tests. In a human cornea model test, undiluted 4-Amino-*m*-Cresol led to a viability of 93.8%; however, the test system was determined not suitable for assessment of the test material because the material was proved to be a strong reducer of MTT reagent. In an ocular irritation study using guinea pigs, 1.5% 4-Amino-*m*-Cresol in 50% propylene glycol was determined to have minimal ocular irritant potential.

The Panel performed an MOE calculation was performed for 0.14% 4-Amino-*m*-Cresol; the MOE was 12,000. This calculation is based on the NOAEL of 60 mg/kg bw/d from a 13-wk oral rat study and an SED of 0.005 mg/kg bw/d. The resulting MOE is greater than 100, which is generally considered to be protective.

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.

Method of manufacturing data and carcinogenicity studies on 4-Amino-*m*-Cresol were not included in the original report and were not found in the updated literature search, and unpublished data were not submitted.

DISCUSSION

In accordance with its Procedures, the Panel re-evaluates the conclusions of previously-issued reports approximately every 15 years. In 2004, the Panel published a final report on 4-Amino-*m*-Cresol and concluded that the available data supported the safety of this ingredient as used in oxidative and nonoxidative (semi-permanent) hair dyes. This report was reopened because several of the other amino-cresol hair dye ingredients that were included in the original 2004 report are banned for use in cosmetics by the European Commission.

The Panel noted that this ingredient functions as an oxidative hair dye in hair coloring products. To estimate risk, the Panel calculated an MOE for 4-Amino-*m*-Cresol using a dermal absorption rate of 0.068% for 0.14% 4-Amino-*m*-Cresol and an NOAEL of 60 mg/kg bw/d. The resulting MOE is approximately 12,000, which is considered protective. (The SCCP calculated an MOE value for 1.5% 4-Amino-m-Cresol in a formulation with hydrogen peroxide (on head) to be 124.4; the SED value used by the Panel is significantly smaller than the one in the SCCP calculation due to a smaller maximum use concentration of 4-Amino-m-Cresol (from 1.5% down to 0.14%) and newly available dermal absorption data (for a concentration of 0.5% in the formulation, which corresponds to 0.425 µg/cm2 (or 0.068% of applied dose)).)

In vitro and in vivo genotoxicity studies yielded mixed results, and no carcinogenicity data were available; however, any concern was mitigated by the weight-of-evidence of negative results for other toxicity endpoints, including developmental and reproductive toxicity, low dermal absorption, and negative results from dermal irritation and sensitization studies. The Panel noted the lack of method of manufacturing information, but data on composition and impurities for this ingredient and the high degree of reported purity obviated this need. The Panel considered these findings, coupled with the short exposure time as a rinse-off product, and determined that the data are sufficient to conclude that 4-Amino-*m*-Cresol is safe for use as a hair dye ingredient in the present practices of use and concentration.

The Panel recognizes that hair dyes containing this ingredient, as coal tar hair dye products, are exempt from certain adulteration and color additive provisions of the Federal FD&C Act when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures. The Panel considered concerns that such self-testing might induce sensitization, but agreed that there was not a sufficient basis for changing this advice to consumers at this time.

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

The Panel's respiratory exposure resource document (available at https://www.cir-safety.org/cir-findings) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be assessed by the Panel. Therefore, the Panel has found the data insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that 4-Amino-*m*-Cresol is safe for use as a hair dye ingredient in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Chemical properties for 4-Amino-*m*-Cresol

Property	Value	Reference
Physical Form	Reddish-brown crystals	2
	Grey powder	6
Molecular Weight (g/mol)	123	2
Density (g/ml @ 20 ºC	1.24	14
Boiling Point (ºC)	not detectable (decomposition)	4
	156 - 190 (decomposition)	5
Melting Point (ºC)	178	2
Vapor Pressure (mmHg @ 20 ºC)	2.48×10^{-5}	4
Water Solubility (g/l @ 20 ºC)	Slightly soluble in water	2
(g/l @ 25 ºC)	2	5
log P_{ow}	0.51	4
UV Absorption (λ) (nm)	maxima at 206, 234, and 300 in ethanol	2
	symmetrical absorption peak at 300	

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
			IN VITRO			
4-Amino-*m*-Cresol; purity not reported	Up to 600 µg/plate without metabolic activation; up to 3000 µg/plate with metabolic activation	ammonia, isopropanol, distilled water	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538	Bacterial reverse mutation test in accordance with OECD TG 471; with and without metabolic activation	Not mutagenic, with or without metabolic activation	[14]
4-Amino-*m*-Cresol; HPLC purity: 97.8 area % (254 nm) and 99.2 area % (300 nm)	1 - 5000 µg/plate	DMSO	*S. typhimurium* strains TA98, TA100, TA102, TA1535, TA1537	Bacterial reverse mutation test in accordance with OECD TG 471; with and without metabolic activation	Not mutagenic; test material did not induce gene mutations with or without metabolic activation; positive control (not reported) yielded expected results	[4]
4-Amino-*m*-Cresol; HPLC purity: 94.7 area %	Test 1: up to 6.25 µg/ml without metabolic activation Test 2: up to 47.5 µg/ml with metabolic activation Test 3: up to 40 µg/ml with metabolic activation	cell culture medium (RPMI with 3% horse serum)	L5178Y mouse lymphoma cells	Mammalian cell gene mutation test at the *tk* locus in accordance with OECD TG 476; with and without metabolic activation	Not mutagenic. In Test 1, slightly increased mutant frequency observed at 3.125 and 6.25 µg/ml; however, a strong cytotoxic effect was observed at 6.25 µg/ml. In Test 2, a slight increase in mutant frequency was measured for 25 and 47.5 µg/ml (no information on cytotoxicity described). In Test 3, no induction of mutants was observed at up to 30 µg/ml and a marginal effect was measured at 40 µg/ml (no information on cytotoxicity described). Negative and positive controls (not reported) were in accordance with the guideline.	[4]
4-Amino-*m*-Cresol (hemisulfate); purity not reported	Up to 5 µg/ml without metabolic activation; up to 1000 µg/ml with metabolic activation	DMSO	L5178Y mouse lymphoma cells	Mammalian cell gene mutation test at the *tk* locus in accordance with OECD TG 476; with and without metabolic activation	Not mutagenic. With metabolic activation, high mutation frequencies observed in one replicate at 125 µg/ml and in both replicates at 1000 µg/ml as compared the solvent control. However, mutation frequencies at other test concentrations (63, 250 and 500 µg/ml) overlapped with the solvent control and regression analysis showed there was no clear dose-related response.	[14]
4-Amino-*m*-Cresol; purity not reported	Up to 20 µg/ml without metabolic activation; up to 156.25 µg/ml with metabolic activation	DMSO	human peripheral lymphocytes	Chromosome aberration test in accordance with OECD TG 473; with and without metabolic activation; negative and positive controls were used	Clastogenic with and without metabolic activation; test material induced significant chromosomal aberrations	[14]
4-Amino-*m*-Cresol (hemisulfate); purity not reported	up to 100 µg/ml with and without metabolic activation	Dulbecco's modified eagle medium/Ham's F12 medium (1:1)	human peripheral lymphocytes	Chromosome aberration test in accordance with OECD TG 473; with and without metabolic activation; negative and positive controls were used	Genotoxic; structural chromosome aberrations were induced, with and without metabolic activation	[14]
4-Amino-*m*-Cresol (hemisulfate); purity not reported	Up to 500 µg/ml without metabolic activation; up to 2000 µg/ml with metabolic activation	anhydrous DMSO	CHO cells	Chromosomal aberration test in accordance with OECD TG 473; with and without metabolic activation; negative and positive controls were used	Clastogenic with and without metabolic activation; test material induced statistically significant and dose-related increases in cultured CHO cells	[14]

Table 2. Genotoxicity studies

Ingredient	Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
4-Amino-*m*-Cresol; purity not reported	Test 1: up to 30 µg/ml without metabolic activation; up to 150 µg/ml with metabolic activation Test 2: up to 35 µg/ml without metabolic activation; up to 125 µg/ml with metabolic activation	DMSO	human peripheral lymphocytes	Micronucleus test in accordance with OECD TG 487; with and without metabolic activation; negative and positive controls were used	With metabolic activation, ambiguous results were observed; statistically higher frequencies of micronuclei binucleate were observed in the 3 highest concentrations when compared to vehicle controls. Single cultures exposed to 100 and 150 µg/ml of test material exceeded historical vehicle control range; however, overall mean micronuclei binucleate frequency fell within historical control range. Without metabolic activation, test material was not genotoxic	14
4-Amino-*m*-Cresol; purity not reported	0.10, 0.33, 1.00, 3.33, or 10.00 µg/ml	DMSO	rat primary hepatocytes	Unscheduled DNA synthesis assay, with metabolic activation only; 2 tests run in parallel; cells treated for 18 h with test material with [^{3}H]thymidine; positive and negative controls used	Ambiguous results. In Test 1, increased nuclear and net grain counts were observed. In Test 2, no reproductible concentration-dependent increase in the number of nuclear grain counts and net grain counts up to the highest concentration were observed.	14
4-Amino-*m*-Cresol (hemisulfate); purity not reported	Up to 500 µg/ml with and without metabolic activation	DMSO	HeLa cells	Unscheduled DNA synthesis assay; with and without metabolic activation; cells treated with tests substance or positive controls plus [^{3}H]thymidine; after incubation for 2.5 h, DNA isolated and quantified	Genotoxic; induced unscheduled DNA synthesis, with and without metabolic activation	14
IN VIVO						
4-Amino-*m*-Cresol; HPLC purity: 94.7 area %	20, 100, or 200 mg/kg bw	0.9% sodium chloride	5 male and 5 female NMRI mice per dose group	Mammalian bone marrow micronucleus test in accordance with OECD TG 474; single intraperitoneal dose; groups of animals killed at 24 h (all doses) or 48 h (200 mg/kg only) post-treatment; appropriate negative and positive controls used. No additional detail was available on the timing of the bone marrow extraction.	Not genotoxic; test material did not induce chromosome aberrations or damage to the mitotic apparatus in bone marrow cells of mice. In all treated groups, the relative polychromatic erythrocyte frequency was not decreased; the highest dose tested induced signs of toxicity (palpebral closure, lethargy) within 1 h of administration. No toxic effects observed at later time points. Positive control yielded expected results.	4
4-Amino-*m*-Cresol (hemisulfate); purity not reported	70, 200, or 600 mg/kg	distilled water	groups of 6 male and 6 female Wistar rats	Unscheduled DNA synthesis assay in accordance with OECD TG 486; rats received single gavage treatment and were killed 14 h post-administration; liver cells isolated and DNA analyzed; appropriate negative and positive controls used	Genotoxic; mean silver grain count was increased significantly compared to negative control in all dose groups; the amount of induced cells in the 200 and 600 mg/kg groups was significantly higher than negative control	14
4-Amino-*m*-Cresol; > 99% purity	60, 600, or 2000 mg/kg bw	DMSO/PEG 400	3 male Wistar/WU rats per dose group	Rat liver in vivo/in vitro unscheduled DNA synthesis assay; single gavage dose; rat hepatocytes studied in vitro after animals dosed in vivo; at 16 h, the 600 mg/kg dose group was killed and at 4 h, the 60 and 1000 mg/kg dose groups were killed; appropriate negative and positive controls used	Not genotoxic. No toxicity observed in any treated animals. No significant induction of unscheduled DNA synthesis compared to negative control group, no significant differences in the viability of hepatocytes in any dose group. Controls yielded expected results. The SCCS noted the study did not meet the requirements of the actual guideline.	4

Table 3. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
IRRITATION						
IN VITRO						
4-Amino-*m*-Cresol; purity not reported	none	undiluted; 10 mg	human epidermal keratinocytes	Skin irritation potential study using the EpiSkin™ reconstructed human epidermis model in accordance with OECD TG 439; 15 min treatment followed by post-exposure incubation period of 42 h; concurrent positive and negative controls utilized	Classified as a non-irritant; test material induced a relative mean viability at 63.3% compared to negative control	[14]
4-Amino-*m*-Cresol; purity not reported	none	undiluted; 25 mg	human epidermal keratinocytes	Skin corrosivity potential study using the EpiDerm™ human skin model in accordance with OECD TG 431; treatment periods of 3 and 60 min; concurrent positive and negative controls utilized	Classified as non-corrosive; test material did not induce decrease of tissue viability; 90.1% viability after 3 min and 80.8% viability after 60 min	[14]
ANIMAL						
4-Amino-*m*-Cresol; purity not reported	0.5% tylose	3% aqueous solution	15 female Pirbright White guinea pigs	Skin irritation study; test material applied daily by brush for 5 d on a 3 cm x 4 cm test area; test sites not occluded but animals were restrained for 5 h post-application; examinations for erythema and edema performed at 5 h post-application	Non-irritating; no skin reactions observed at any observation time point; no clinical signs of toxicity observed	[4]
SENSITIZATION						
ANIMAL						
4-Amino-*m*-Cresol; purity not reported	distilled water	induction: 3% challenge: 1, 2, and 3%	test group: 20 Pirbright Hoe:DKPK guinea pigs control group: 10 guinea pigs	Guinea pig maximization test; first intradermal induction with 0.05 ml of test material in distilled water with Freund's complete adjuvant/arachidis oil injected in parallel; after 6 to 8 h, animals pre-treated with 10% sodium lauryl sulfate in pet. and topical induction with 5 ml of test material in pet. on 2 cm x 4 cm test area on flank, test site occluded for 24 h; second intradermal induction with test material in Freund's complete adjuvant/arachis oil; challenge phase started on day 16 of study with patches of 0.5 ml of test material in Freund's complete adjuvant/arachis oil under occlusion for 24 h; test sites evaluated after 24 and 48 h; concurrent vehicle and positive (1-chloro-2,4-dinitrobenzne) control groups were used	Non-sensitizing; no dermal irritation observed during the induction phase and no dermal sensitization observed during the challenge phase; controls yielded expected results The SCCP noted the test design of this study was inadequate and had reporting deficiencies	[4]
4-Amino-*m*-Cresol; 95.8% pure	DMSO or aqua/acetone (1:1) mixed with olive oil (4:1)	DMSO: 0, 0.5, 1.5, 5.0, or 10% water/acetone/oil: 0, 0.5, 1.5, 3.0, or 5.0%	groups of 5 female CBA/J mice	LLNA; mice received test material (25 µl) on ear surface once daily for 4 d; p-Phenylenediamine (1%) in DMSO was positive control; 5 d after first topical application, all animals were injected intravenously with [^{3}H]methyl thymidine and the proliferation of lymphocytes in the draining lymph nodes was measured	Moderate sensitizer; no abnormal signs of toxicity or mortality during study. Mean SI were calculated to be 0.9, 3.1, 6.5, and 6.7 for the 0.5, 1.5, 5.0, and 10% dose groups in DMSO, respectively; estimated concentration for the EC_3 was calculated to be 1.45%. For the water/acetone/olive oil groups, the SI were calculated to be 1.5, 1.7, 4.7, and 6.9 for the 0.5, 1.5, 3, and 5% dose groups, respectively. EC_3 for this group was calculated to be 2.15%. Controls yielded expected results.	[4]

Table 4. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
IN VITRO						
4-Amino-*m*-Cresol; purity not reported	50% propylene glycol	1.5% (w/w) dilution; 30 µl	3 eyes from Ross spring chickens	Isolated chicken eye test method in accordance with OECD TG 438; eyes exposed to single application of test material for 10 s and observed for up to 4 h; concurrent positive and negative controls utilized	Classified as not irritating	[14]
4-Amino-*m*-Cresol; purity not reported	none	undiluted; 30 mg	3 eyes from Ross spring chickens	Isolated chicken eye test method in accordance with OECD TG 438; eyes were exposed to a single application of test material for 10 s, rinsed with 20 ml saline, and observed for up to 4 h; concurrent positive and negative controls utilized	Classified as not irritating; test material did not cause corneal effects other than very slight corneal swelling; microscopic examinations of the corneas did not reveal any abnormalities	[14]
4-Amino-*m*-Cresol; purity not reported	none	undiluted; 50 mg	human reconstructed cornea	Eye irritation potential test using the human cornea model test in accordance with OECD TG 492; tissue exposed for 6 h; concurrent positive and negative controls utilized	Test system not suitable for assessment of test material; the test material led to a viability of 93.8%; however, the test material was proved to be a strong reducer of MTT reagent	[14]
ANIMAL						
4-Amino-*m*-Cresol; purity not reported	50% propylene glycol	1.5%; 0.1 ml	5 female Pirbright white guinea pigs	Ocular irritation study; test material instilled in the conjunctival sac of the left eye; eye was not rinse; right eye served as control; ocular reactions recorded at 0.5, 1, 2, 3, 4, 6, and 7 h after instillation; further reading by fluoresce-instillation occurred at 24 and 48 h post-instillation	Minimal ocular irritation potential; conjunctival erythema observed in 1 animal without other macroscopic effects; no ocular irritation observed in remaining animals	[4]

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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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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Monice Zondlo Fiume and Torill A. Yamarik prepared this report. Address correspondence to F. Alan Andersen, PhD, Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 310, Washington, DC 20036, USA.

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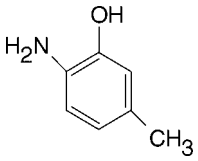
used in hair tints and hair dyes and colors at concentrations of ≤1%, ≤5%, and ≤1%, respectively.

CHEMISTRY

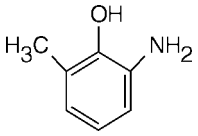
Definition and Structure

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



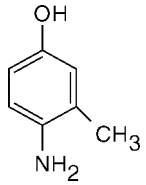
6-Amino-*m*-Cresol is also known as 4-Amino-3-Hydroxytoluene; 2-Amino-5-Methylphenol; Phenol, 2-Amino-5-Methyl-; 2-Hydroxy-4-Methylaniline (Pepe, Wenninger, and McEwen 2002); *m*-Cresol, 6-Amino; 6-Amino-3-Cresol; 6-Amino-3-Methylphenol; 2-Hydroxy-*p*-Toluidine; 5-Methyl-2-Aminophenol (Regulated Chemicals 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):



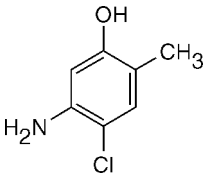
6-Amino-*o*-Cresol is also known as 3-Amino-2-Hydroxytoluene; 2-Amino-6-Methylphenol; Phenol, 2-Amino-6-Methyl-; 6-Amino-2-Methylphenol; Phenol, 6-Amino-2-Methyl-; 2-Hydroxy-3-Methylaniline (Pepe, Wenninger, and McEwen 2002); *o*-Cresol, 6-Amino; 6-Methyl-2-Aminophenol (Regulated Chemicals Listing 1998); 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):



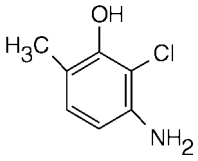
4-Amino-*m*-Cresol is also known as 2-Amino-5-Hydroxytoluene; 4-Amino-3-Methylphenol; Phenol, 4-Amino-3-Methyl-; 4-Hydroxy-*o*-Toluidine (Pepe, Wenninger, and McEwen 2002); 3-Methyl-4-Aminophenol (James Robinson Ltd. 1998); *p*-Amino-*m*-Cresol; *m*-Cresol, 4-Amino-; 4-Hydroxy-2-Methylaniline; *p*-Hydroxy-*o*-Toluidine; *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:



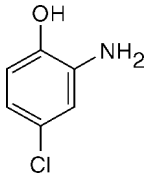
5-Amino-4-Chloro-*o*-Cresol is also known as 5-Amino-4-Chloro-2-Methylphenol; Phenol, 5-Amino-4-Chloro-2-Methyl- (Pepe, Wenninger, and McEwen 2002); and 2-Methyl-4-Chlor-5-Aminophenol (Henkel KGaA 1994).

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:

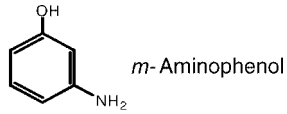


4-Chloro-2-Aminophenol is also known as 2-Amino-4-Chlorophenol; Phenol, 2-Amino-4-Chloro-; 2-Hydroxy-5-Chloroaniline; CI 76525 (Pepe, Wenninger, and McEwen 2002; Regulated Chemicals Listing 1998); 5-Chloro-2-Hydroxyaniline; *o*-Amino-*p*-Chlorophenol; *p*-Chloro-*o*-Aminophenol; and C.I. Oxidation Base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

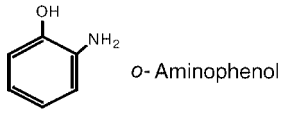
TABLE 1
Physical and chemical properties of 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol (Henkel KGaA 1994, 1996)

	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)

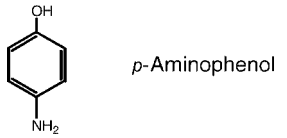
Structure of Related Ingredients



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

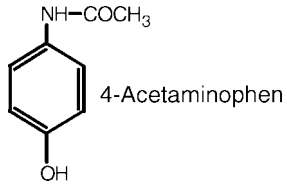
No data were available on ingredients in this safety assessment, but data on related ingredients are summarized below.

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.

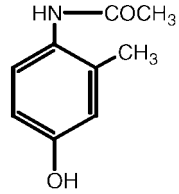
Acetaminophen

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).

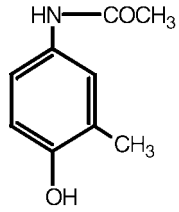


4-Acetaminophen

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.

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