Amended Safety Assessment of 6-Amino-*m*-Cresol as Used in Cosmetics

Status:	Draft Tentative Amended Report for Panel Review
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Panel Meeting Date:	June 12-13, 2023

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. This safety assessment was prepared by Christina Burnett, M.S., Senior Scientific Analyst/Writer, CIR.

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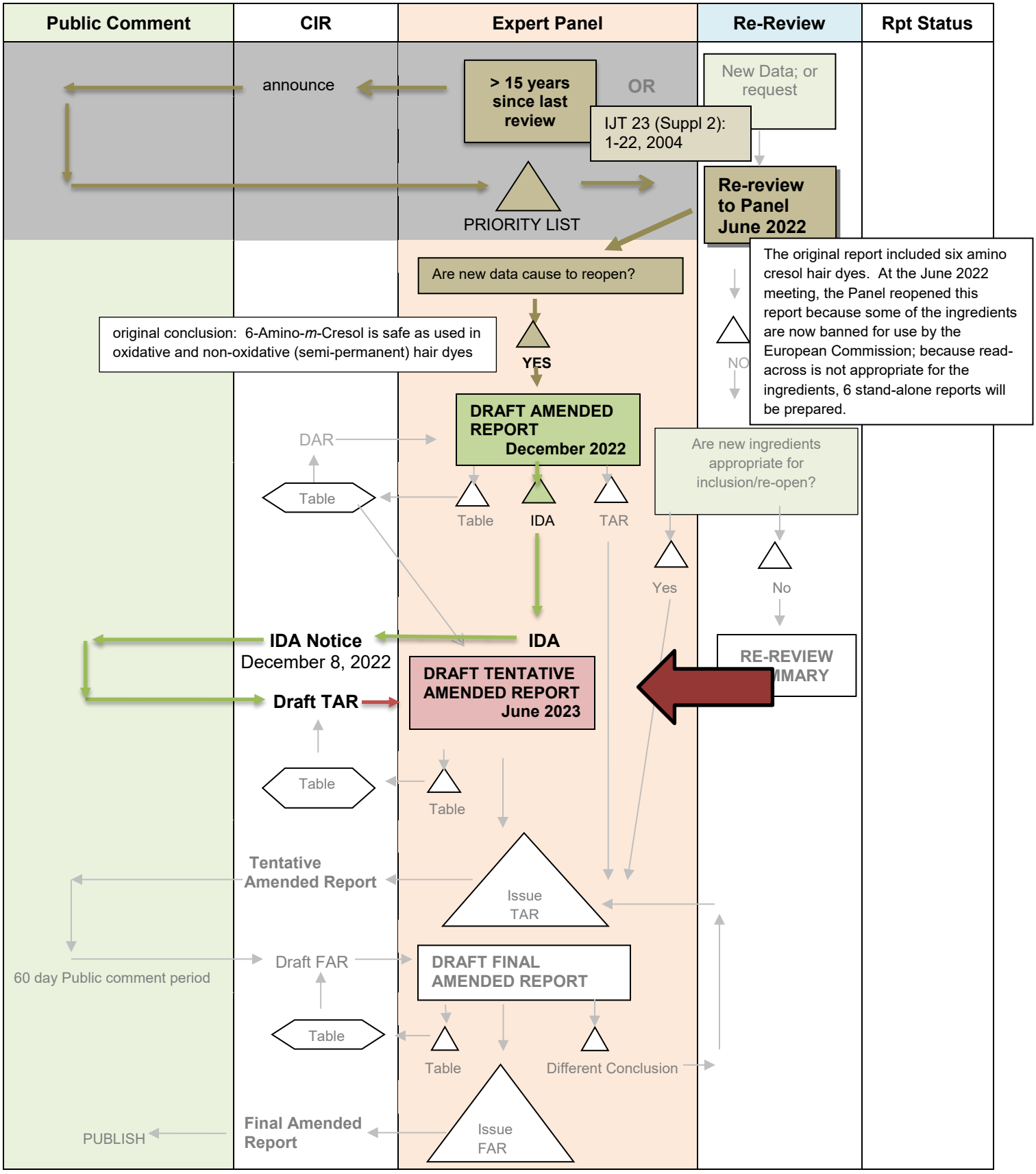
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY 6-Amino-*m*-Cresol

MEETING June 2023



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: May 19, 2023
Subject: Amended Safety Assessment of 6-Amino-*m*-Cresol as Used in Cosmetics

Enclosed is the Draft Tentative Amended Report on the Safety of 6-Amino-*m*-Cresol as Used in Cosmetics. (It is identified as *report_6-Amino-m-Cresol_062023* in the pdf document). At the December 2022 meeting, the Panel determined that the data were insufficient to support safety of this hair dye ingredient. The additional data needs are:

- Method of manufacture
- In vivo genotoxicity studies

Since the Insufficient Data Announcement (IDA), CIR has received no new data. The 2023 VCRP survey data report there are no uses for this ingredient; last year, there were 2 uses in hair dyes. However, please note that concentration of use data (0.69% in hair dyes and colors) were submitted in response to the Council survey, which indicates at least one use. Comments that were provided by the Council prior to the December 2022 meeting have been addressed.

The relevancy of the airbrush boilerplate language in hair dye reports has been questioned and a request was made to remove the boilerplate language in the Use section of this report. While this type of use is not reported in the VCRP or in the Council's concentration of use survey, CIR staff has been made aware that airbrush application of hair dye products are being advertised and sold on the Internet. The Panel should discuss whether the airbrush boilerplate language should continue to be added to hair dye reports or discontinued.

Additional supporting documents for this report package include the original report (*orginalreport_6-Amino-m-Cresol_062023)*, flow chart (*flow_6-Amino-m-Cresol_062023*), report history (*history_6-Amino-m-Cresol_062023*), a search strategy (*search_6-Amino-m-Cresol_062023*), a data profile (*dataprofile_6-Amino-m-Cresol_062023*), transcripts from the meeting at which the re-review was discussed (*transcripts_6-Amino-m-Cresol_062023*), and the minutes from all the meetings at which 6-Amino-*m*-Cresol was discussed during the original review (*originalminutes_6-Amino-m-Cresol_062023*).

A draft Abstract and Discussion have been included in this report version. The Panel should carefully consider and discuss the data (or lack thereof), and issue a Tentative Amended Report with a safe, safe with qualifications, insufficient data, unsafe, or split conclusion, and identify any additional items for inclusion in the Discussion.

6-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 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 6-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 it being banned for use in cosmetics by the European Commission.

December 2022 – The Panel issued an Insufficient Data Announcement (IDA) for 6-Amino-m-Cresol. The additional data needed to determine safety for this hair dye ingredient are:

- method of manufacture
- in vivo genotoxicity studies

6-Amino-*m*-Cresol Data Profile* – June 2023 – 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
6-Amino-*m*-Cresol	XO		X	X	X	X		X O			X O			O	XO	XO				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.

6-Amino-*m*-Cresol

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
6-Amino-*m*-Cresol	2835-98-5	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Search Strategy (from 2002 on)

PubMed

((“6-Amino-m-Cresol”) OR (2835-98-5[EC/RN Number])) - 10 hits; 0 relevant

ECHA

No dossier for CAS #2835-98-5

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.

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?

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 - 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.

DECEMBER 2022 PANEL MEETING – DRAFT AMENDED REPORT

Belsito's Team – December 5, 2022

DR. BELSITO: Moving onto another hair dye. Are you the hair dye queen?

MS. BURNETT: I am the hair dye queen.

DR. BELSITO: Okay. 6-Amino-m-Cresol. So, we heard that the M's are usually developers that are allowed that make it a trimer; is that right, if I followed all that chemistry?

DR. SNYDER: You were listening.

DR. BELSITO: I was listening.

DR. RETTIE: I don't think it necessarily has to be a trimer. It's a coupler, right?

DR. BELSITO: We also got Wave 3 comments. Have you had a chance to look at those, Christina?

MS. BURNETT: Yeah, they're editorial. Yeah.

DR. BELSITO: Okay. So, you'll just incorporate all of those?

DR. SNYDER: Wave 2, we have the genotox data as positive. We had it all negative before, but Wave 2, we have some positive.

DR. BELSITO: Yeah. So, I didn't understand why, Christina, in the use section, you just carried over that airbrush because it's not going to be relevant for a hair dye. Could we just delete all of that in the cosmetic use section?

MS. BURNETT: For this report, yes. Let me make sure. For one of them, it has aerosol use.

DR. BELSITO: How could a hair dye have aerosol use?

MS. BURNETT: Spray hair dye.

DR. BELSITO: Spray dye. Okay.

MS. BURNETT: Like a Halloween costume spray.

DR. BELSITO: Oh. Okay. Well, if it does --

MS. BURNETT: We'll get to that one. It's not this one, it's Basic 87, I guess. If you don't believe it's needed for this report, we can take it out.

DR. BELSITO: I just assumed -- I didn't even look to see if there were spray uses, I just assumed that -- wow, that's interesting stuff. Never go on assumption, huh. Where's the uses?

MS. FIUME: I think it's -- there's some text. That use are (inaudible) text?

MS. BURNETT: I believe so, yeah.

DR. BELSITO: Two reported uses in hair dyes and colorants. It doesn't say whether it's in a spray or not.

MS. BURNETT: It's not this one. This one is just straight hair dye use.

DR. BELSITO: Okay. Then I don't think we need all the aerosol stuff.

MS. BURNETT: Okay.

DR. BELSITO: So, I guess the first question that I had for Allan and Curt are the impurities that are listed here. Given the low concentration and the impurities and the low final dilution of rinse-off, I'm assuming you're not concerned with those?

DR. RETTIE: I've got a note, impurities: 99 percent plus over four batches.

DR. BELSITO: Yeah.

DR. RETTIE: There's no method of manufacture, but -- we just knew this by distillation of coal tar and just move on?

DR. BELSITO: I thought the absorption data on page 19, PDF 19, .58 percent of the applied dose of the test material in the hair dye formulation, sort of with the predominant negative genotoxicity.

DR. SNYDER: No, that table. You got to go to that table, Table 2 on Page 24. It's all positive. All the uses. It's all positive now. In the old report it was all negative, now we got the whole table of all positive genotox. Page 24.

DR. BELSITO: I haven't gotten there yet. I say, team, what to make of these? And what contradictory studies does in vivo test trump in vitro? Was it just the in vitro, Table 4, you said?

DR. SNYDER: No, both in vitro and in vivo, both, are positive now.

DR. BELSITO: Yeah. But in the old ones --

DR. SNYDER: It's all negative.

DR. BELSITO: -- it was all negative.

DR. SNYDER: I don't know how much was in that old report, I looked, but --

DR. RETTIE: There's also a dimer product that's -- I've got a note here it's absorbed in human skin, in vitro. I didn't get to read any VEC report, so I don't know how much. That's an issue.

DR. BELSITO: We have sub-chronic oral in the old report. Wait a minute, we're mixing apples and oranges because the old report contained a whole bunch of other cresols. And we're looking at just 6-Amino-m-Cresol.

DR. SNYDER: Oh, you're right. Yeah.

DR. BELSITO: What was the genotox data for 6-Amino? 6-Amino-m-Cresol, we had --

MS. FIUME: Christina has it summarized in italics.

MS. BURNETT: PDF, Page 21.

DR. BELSITO: So, there's toxicity going on there, too.

DR. SNYDER: Yeah.

MS. BURNETT: There were more in vivo tests in the original report that were negative; whereas, there's one positive in vivo in this new report.

DR. BELSITO: Yeah. So, that's why -- my comment was actually on your summary, what to make of these and what contradictory studies, when you add the old report with the new report.

DR. SNYDER: I don't know if Tom can glean something that's different in methodology or whether the cell depth matter than the old report. I mean, the old assays or --

DR. BELSITO: Curt, any feelings about the difference we're seeing the genotox data now than compared to the old?

DR. KLAASSEN: Okay. It's very concerning in why there should be such a difference. I think, as was mentioned, let's see what happens tomorrow and see what Tom thinks, if he can figure out a reason for this, but this is not good.

DR. SNYDER: Is there a difference, a large difference, in maximum amount put on the plates in Ames? I see 1000 micrograms per plate in the italics text, in the old data, and I see 5000 micrograms per plate, maximum on the new data. So that's an increase of five-fold. Would that be enough to change this?

DR. KLAASSEN: It could be.

DR. BELSITO: It could change the toxicity for sure.

DR. SNYDER: Well, it says it was toxicity noted in 2500 and above.

DR. RETTIE: Which is still higher than the original 1000.

DR. BELSITO: Okay. So, we're going to punt this to Slaga; is that it?

DR. SNYDER: What we're concerned about, yes. We just want to see if there is a reason that -- if there's a justification as to why.

DR. KLAASSEN: I think Tom has a question.

DR. SNYDER: Go ahead, Tom.

MR. GREMILLION: I noticed this one, the SCCS in 2012, determined that it was not safe for consumers when using an oxidative hair dye formulation with a concentration of 1.5 percent. I know the reported uses here is a little under that, but I wondered, you know, if the data in here countered some of the concerns that were presented in that report.

I also wondered about, on page 22, the study where the NOAEL couldn't be calculated. Is that just a reflection of the study design? That seems like a red flag. But I take it that your attention has already been drawn to some of these positive genotoxicity studies.

DR. BELSITO: Tom, what page are you on because 22 is a summary.

DR. SNYDER: He's talking about the 90-day oral gavage study, where they didn't have any histopathology or macroscopic, but they had feed consumption, body weight changes that were reduced in both sexes at all doses. So, that's why they couldn't come up with a NOAEL.

MS. BURNETT: Bottom of page 20.

DR. BELSITO: Oh, okay. Right. So did you hear, Tom, what Paul said? Basically, is they didn't look at tissues so --

DR. SNYDER: No, they did. There was no effects on the gross or the microscopic, it was only the changes in the relative and absolute liver, kidney and spleen weights were increased.

DR. BELSITO: Right.

DR. SNYDER: And body weights were decreased. So, that's why they couldn't come up with it.

MR. GREMILLION: That's a sign of pathology.

DR. SNYDER: Correct. There was nothing to correlate to those indicating toxicities.

DR. BELSITO: Right. They just had weight changes. So, they had increased weight of organs, decreased weight of the body, but when they looked at it microscopically, and histopathologically, they saw nothing to account for it, which is sort of weird, but.

DR. SNYDER: It happens.

DR. BELSITO: Okay. So, I think where we're with this is that our only concern is the new genotox data. We don't know how to interpret it, particularly because of cytotoxicity issues and increased concentrations that were done with these newer tests. And we're going to ask Tom -- we're going to hear what Tom thinks about it because I just checked, and the Cohen team is going to report on this. So, we'll get to ask -- or hear what Tom felt about this.

Anything else? It was really to genotox on this, right?

DR. SNYDER: Yes.

Cohen's Team – December 5, 2022

DR. COHEN: Of course. Okay, so the panel previously reviewed the safety of 6-aAmino-*m*-Cresol, 6-Aminio-*o*-Cresol, 4-Amino-*m*-Cresol, 5-Amino-4-Chloro-*o*-Cresol, 5-Amino-6-Chloro-*o*-Cresol, and 4-Chloro-2-Aminophenol in 2004. In

June, we reopened the safety assessment due to the fact that some of these hair dyes were banned in Europe and the panel didn't feel that we could read across on all of those. So, all six ingredients will be rereviewed as standalone reports.

According to the most recent VCRP, 6-Amino-*m*-Cresol has two reported uses in hair dyes and color and that came up this morning during the lecture. At the time the report was issued, the max use was at 2.4 percent in hair dyes and colors. Now the reported concentration is down to 0.69 in the reports. Since the June meeting, no new data has been submitted. And we have structure and impurities, we don't have method of manufacturing in this report.

We have absorption. So, what's your opinion on how we adjudicate with this report? David, yeah, I'll start with you.

DR. ROSS: I wish you hadn't started with me.

DR. COHEN: I know, yeah. I didn't want to start so.

DR. ROSS: Well, here we go. Wanted Tom's opinion of this anyway. But when I looked at this, I just assumed all the genotox data in here would've been from the previous reports. So, I went straight to the previous report and all the genotox in that report was solid and it was a good conclusion. And all the genotox data in the 2004 report was negative. And so, then I went to our dossier and I'm looking at it and thinking, this doesn't match because there's a lot of positives in this new genotox data.

All the new genotox data is either -- it's positive in every assay, with either metabolism or not metabolism. So, there's some condition that's positive and then there's also a positive micronuclei test in vivo. So, I think the issue for me was the new genotox data. And we do have purity data with this compound, so that's good.

But the genotox data now looks to me as a significant concern, and I think that was the European conclusion if I recall, which was done I think in 2012, Bart, does that sound right? But anyway, yeah. So, for me it was the new genotox data. And I want advice from my learned colleagues on the panel, how to deal with that.

DR. COHEN: Tom? Going right to Tom.

DR. SLAGA: Well, as a separate report. I would go with safe.

DR. COHEN: Any comments on Dave's concern about the inconsistencies from one year to another on the genotox?

DR. SLAGA: No, I didn't have -- I think the weight of evidence is okay.

DR. COHEN: I think if I heard him right, didn't Carsten say that this was metabolized to non-genotoxic components?

DR. ROSS: Well, I mean, you still -- from what I got from this morning's presentation, the precursors are still going to be around.

DR. COHEN: Yes.

DR. ROSS: And so, precursors we're worried about, not necessarily the reaction products. There was a comment in the European opinion on the reaction products, but I'm not too concerned about that, given this morning's presentation. It's a precursor, the Cresol that I'm concerned about.

DR. COHEN: Susan?

DR. TILTON: So, I had noted similar concerns as David about the new evidence of genotoxicity and mutagenicity both with and without metabolic activation. So, it was specifically in terms of the recommendation for the direct or semipermanent formulation.

In terms of its use in oxidative formulations, it still seems like there is, as David just mentioned, some of these precursors available. They're not fully oxidized. And so that was where I primarily had a question, was about that formulation, the use of these in the permanent oxidative form.

DR. ROSS: So, could we -- just an idea. I mean, I don't have it clear in my mind how to formulate this conclusion, but could we argue that under conditions where the precursor is removed quickly, that it's reasonable where it's not. Because we do have purity. We know what we're putting on the head so it's pretty pure.

DR. BERGFELD: You said that's pretty quick, that precursor availability?

DR. COHEN: In the oxidative --

DR. BERGFELD: Yeah.

DR. COHEN: -- model?

DR. ROSS: You know, that's what I'm a little confused about with -- you know, I think there's a conclusion in the next one we have coming up with which is the ortho product, which differentiates the oxidative and the non-oxidative. But

given the presentation this morning, I'm not sure that conclusions is entirely correct. But if we could somehow do that with this, the better.

DR. COHEN: I don't think that's a very easily operational conclusion for anybody. So, your thoughts on that, in the oxidative hair dyes there's going to be a lot of precursors still floating around. But why would that -- and in the non-oxidative hair dyes it doesn't?

DR. ROSS: I suppose that's what I'm asking you.

DR. BERGFELD: Does it have to do with the amount of peroxide in it? Hydrogen peroxide?

DR. ROSS: The presentation you got this morning was the reaction of the precursor with peroxide was relatively slow, and that was the first step. So that was my notes on it. So, it's going to be around, the precursor.

DR. COHEN: But for minutes, right?

DR. ROSS: I don't know for how long. I mean, there's reaction conditions with --

DR. BERGFELD: Do you have that? You have that lecture --

DR. ROSS: I think it was 30 minutes. I think the reactions were 30 minutes.

DR. TILTON: There was still some detected after 30 minutes.

DR. COHEN: There was.

DR. TILTON: Yeah. So, it was not fully oxidized.

DR. HELDRETH: Yeah, the general notion that I got reading through his presentation, and his presentation that he made back in 2017, was that although you would form your dye product, most of that is trapped in the hair. And so exposure to the dye product is actually relatively small to the consumer.

But the precursor and the coupler do not affix to the hair, and what remains around is going to be what the consumer is exposed to. So, the precursor and the coupler are probably the most prominent two pieces, whether it's oxidative or not.

DR. ROSS: Yeah, okay. So, we can't really differentiate that as David is suggesting.

DR. COHEN: But those are mixed, right?

DR. HELDRETH: They're mixed.

DR. COHEN: And then put on?

DR. HELDRETH: That's right. But the coupler and the precursor are not consumed fully.

DR. COHEN: Right. There's a fair amount that's -- we saw that in the 30 minutes, there was still precursor sitting around and then the products were starting to accrue.

DR. HELDRETH: Right.

DR. TILTON: I mean, and that's when -- that's where the toxicokenetics can come into play and the extent on metabolism. But from the report, my understanding that while this is highly -- extensively metabolized orally, it's metabolized less so dermally. Although our speaker today said that it was rapidly metabolized in the skin, I believe. So, maybe there's other data to support that. It's different than what was in this report.

DR. COHEN: Tom, having heard all that, how -- when I present this tomorrow, if I go --

DR. SLAGA: I couldn't hear the last speaker.

DR. COHAN: Susan, can -- that's just the recorder, I think, right?

DR. TILTON: Oh, okay. Can you hear me now? My computer may have been in my way. So, we had just been talking about the fact that the precursor products are not fully oxidized in the oxidative reactions.

And so, then talking about the extent of metabolism in the skin, and the fact that the current report indicates that dermal metabolism -- it's extensively metabolized orally but less so dermally. Although our speaker today seemed to suggest that there was also extensive metabolism in the skin, and we may not have that information in this report.

DR. COHEN: So, Tom, if we go out with safe as used as a hair dye here, and tomorrow all of these genotox reports will come back for discussion and as residual precursor in the hair and they are -- they seem to have positive genotox to them, how do we justify that?

DR. SLAGA: Can't justify that without more information on that. It is a draft amended report, we could ask for anything we want. It's not a necessity to go forward. I was basing it on how we reviewed it last time for the hair dye.

This particular ingredient, there were several other physicians that had some concern, or commissioned it. Let's ask for whatever we need to try to satisfy it.

DR. COHEN: So, what are those questions, if we go out with an IDA?

DR. SLAGA: Yeah. That'd be fine.

DR. TILTON: I mean, previously dermal carcinogenicity had been requested and it doesn't seem like that's been provided.

DR. ROSS: When I looked at this, because of that genotox data, following up on Susan's comments, you know, I've got a note here that says may be insufficient to determine safety in the absence of carcinogenicity data. You know, again, I just keep coming back to this genotox. I mean, I wouldn't be happy with a safe as used with this one.

DR. COHEN: So is our only -- we go out with an IDA, and we may get an answer or not get an answer. Is the only thing we're asking for is dermal carc. now? Because now's the time to ask for everything we want. What else do we want?

DR. HELDRETH: You're also missing method of manufacture, right?

DR. COHEN: Yeah. Let me add that.

DR. ROSS: That was one --

DR. COHEN: Anything else, guy- -- I mean we can go out with the IDA, we can ask for dermal carcinogenicity. If the dermal carcinogenicity came back negative, would we go out with the safe as used as a hair dye?

DR. SLAGA: You're asking me if we could get carcinogenicity, I doubt it?

DR. COHEN: No. I'm fast-forwarding to the future. We have an IDA and then we ask for material, and we get it. I'm asking the group, if dermal carc. came back negative would we then issue a safe as used statement? Would the rest of the panels say yes to that?

DR. ROSS: Could I go back one question?

DR. COHEN: Sure.

DR. ROSS: Are any of the things that we wanted to ask for? I mean, the genotox which worries me most is the new in vivo assay which I think was micronuclei. It would be nice to have more in vivo genotox on this compound. I mean, that might be easier to get than dermal carcinogenicity. And whether it's unscheduled DNA synthesis, whether it's something else, in vivo might give us more direction. Does that make sense?

DR. COHEN: Yeah. I mean, that makes sense. Tom, would that be helpful for you?

DR. SLAGA: Yeah. That would be fine, yeah.

DR. COHEN: Susan?

DR. TILTON: Yes. I agree.

DR. COHEN: So, if we got that, I mean, we'd have to fast-forward to if we actually get what we ask for, are we prepared to adjudicate this one way or the other? And if this is the information we want, we'll go with it. We didn't have time today but when I reviewed this one, I was looking back at the report and we lean on the fact that all of the hair care products in the US suggest an allergy alert test, right. And I dug into that because I speak about it all the time at my lectures because it's a very practical approach to dealing with patients.

And it turns out that there's no common protocol for the allergy alert test. So, FDA's website lists applying the product and leaving it on for 48 hours. WELLA suggests applying the product and rinsing off after 45 minutes. And there's actually a study that looks at the question about that and there's no uniformity. About a third say leave it on for 48 hours, a third say leave it on for 45 minutes, and a third mention nothing about rinsing at all. And that occurs even within the same company making different hair dye products.

And of course, there's a comment in here about the concern that people have raised about this being a sensitizing event, by continuously applying the product to the skin. I think probably applying it for 48 hours, it's like a slow motion HRIPT if you're doing it every month or every three weeks. So how do we get some uniformity? I have always used the 45-minute rule. Loreal uses a 48-hour rule, the FDA uses a 48-hour rule. How can we harmonize this because it's rather important.

DR. BERGFELD: You can't.

DR. COHEN: Though we can comment -- we have freedom to comment in our reports on it, right?

DR. BERGFELD: Yeah. And you put it in the minutes, and you can ask FDA to comment on it. It's up to them to horse this around and make it harmonious. We just know that that patch test is not usually done clinically.

DR. COHEN: Yeah.

DR. BERGFELD: And that everybody knows about it, the client and the artist that's doing their hair, but they don't abide by the rule.

DR. COHEN: But we lean on it on a report, right, and that's the quandary, right?

DR. BERGFELD: That's all we can do.

DR. COHEN: I think people are more apt to try to do it, or get advice to do it, if they're running into an issue.

DR. BERGFELD: Yes.

DR. COHEN: Right. So, I don't think it's never used, I just don't think it's used by people who have no problem with it.

DR. BERGFELD: Majority.

DR. COHEN: The majority. Right. So, can that go in the discussion at all or it's a little heady for that? That there's great variability?

DR. BERGFELD: There's no documentation. Do you have documentation of it?

DR. COHEN: We do. There's peer reviewed articles about it.

DR. BERGFELD: Okay. Probably can then.

DR. COHEN: I got them. I'll send them.

DR. HELDRETH: Yeah, and if we will be putting that in our discussion, and we have citations, then we'll want to mention it somewhere probably in the cosmetic use section. Because we typically try not to do any citations in our discussion section. It's supposed to be referring back to -- with them.

DR. COHEN: Yes.

DR. HELDRETH: So, maybe present that information, a short blurb, somewhere in the Use section, with the references, --

DR. COHEN: Ah, that's perfect.

DR. HELDRETH: -- and then explain the panel's rationale for including it in the discussion.

DR. BERGFELD: I have a question to ask just about penetration. These dyes are applied, and I have a blonde who dyes her hair 30 minutes, and rinsed off. But is there a time on the penetration? I didn't catch that in the -- you saw that? That Y and X --

DR. COHEN: Yeah, it looked like it occurs pretty quickly.

DR. BERGFELD: Quickly.

DR. COHEN: Early on and then it falls off.

DR. BERGFELD: In the skin studies?

DR. ROSS: Yeah.

DR. COHEN: Yeah, I think that was the France cell chamber stuff. Right, the in vitro? Right? And then with the serum levels also were very shifted to the left. It was very --

DR. BERGFELD: Quick.

DR. COHEN: -- quick peak and then down. Yeah, all the action's happening during the process.

DR. BERGFELD: Yeah, the John Corbett (phonetic) who is mentioned there presented at some of the first CR meetings on hair dyes. And he would always talk about the quickness of the reaction and the fact that there is very little residue left or agents left. Do you know his name, John Corbett?

DR. HELDRETH: It's familiar.

DR. ROSS: I think it was referenced this morning, yeah. Yeah. Yeah. That wasn't what we got this morning. It was a different conclusion.

DR. COHEN: I think another -- I'm trying to figure out where this would go. I don't know how much the standard deviation that they built into the exposure model actually reflects the real-life use of the product at home, right.

DR. BERGFELD: Well, I think, when you said that at home they massage it in. You know, at the hairdressers they also massage in. So, I'm not sure there's a big difference and I don't know of any studies that would show that.

DR. COHEN: No, I don't think so.

DR. BERGFELD: They've had , actually, with the home use they've had less trouble. I remember that from Corbett, that the home use was not worse outcomes for adverse events than the salon use.

DR. COHEN: But we have such reporting problems.

DR. BERGFELD: Right.

DR. COHEN: -- with this. Right? I think we need to make a com- -- well, do we comment in the report or do we comment amongst ourselves that the VCRPs not great for this?

DR. BERGFELD: You can certainly comment in the minutes.

DR. HELDRETH: Right. Yeah, frequency of use is particularly poor when it comes to the hair dye ingredients for estimating how many people are exposed to it. Because of the nature of it, you know, we don't just sell this as a formulation. We sell it as a formulation that gets mixed with the coupler and with the peroxide. And so, 25 uses in an VCRP may represent many more consumers than 25 uses of, say, of, a skin conditioning agent.

DR. COHEN: Would a hair -- you know the dye colors -- the dye products have very interesting names and within them there could be 25 colors. So, is that one VCRP use or is that 25?

DR. TILTON: I'm sorry, I don't follow.

DR. BERGFELD: The mixture, he's talking about.

DR. COHEN: In other words, Wella Pulse and Perfect Innocence. Right, is that one use if they have 20 plus colors?

DR. HELDRETH: The frequency of use is ingredients.

DR. BERGFELD: To get the color it's 25 different chemicals.

DR. HELDRETH: The VCRP frequency of use is ingredient specific. So, when we say there's a certain number of uses for this 6-Amino-*m*-Cresol, it's just for that ingredient.

DR. COHEN: Right. In a product?

DR. HELDRETH: In a product.

DR. COHEN: Right. But this -- does all the colors represent different boxes in the store, that's one use or is that all of those?

MS. BURNETT: No, those are all separate. Yeah.

DR. COHEN: Yeah, then it's, you know, it's really hard to use the VCRP here.

DR. HELDRETH: Right.

MS. BURNETT: Yes. Especially like, your hair dye it might be six uses but maybe those are the most popular -- you know, it's a very specific color that's very popular so if it has the most sales or something.

DR. HELDRETH: Right.

MS. BURNETT: So, it could reach more people or just the same amount of people as if it was used in more products.

DR. HELDRETH: Right. And that's why we make sure to prioritize at least one hair dye each year, because we can't rely on the frequency of use to give us a rough estimate of how many consumers are exposed.

DR. COHEN: Do we make some commentary in the reports about the target of these products are the hair, not the scalp or the face and neck? Because we certainly make a comment about the eyebrows and the eyelashes. It just goes to the absorption issues. The scalp's very vascular.

MS. BURNETT: I can't remember if we changed the wording over the years. We might've, at one point in time, to minimize contact.

DR. COHEN: Yeah, that's kind of what I'm getting at.

MS. BURNETT: I know on the packaging they tell you to minimize contact. You know, to wipe off as soon as possible. If it gets on your skin, they suggest putting petroleum jelly around the scalp, the hairline. Again, I don't know how well that's practiced -- myself, no -- but you know.

DR. COHEN: No. This comes up in the context of people having issues.

MS. BURNETT: I know.

DR. COHEN: Right. Okay. So, we're going to go on to summarize an IDA asking for dermal carc., method of manufacturing and more in vivo genotoxicity. We have micronuclei studies, but we'd like some others.

DR. ROSS: Yeah. Correct. In vivo genotox. Yeah.

DR. COHEN: Good. All right.

DR. BERGFELD: So, you have three items that you're requesting?

DR. COHEN: I'm sure this'll be heavily discussed tomorrow.

Full Panel Meeting – December 6, 2022

DR. COHEN: The Panel previously reviewed the safety of 6-Amino-m-Cresol, 6-Amino-o-Cresol, 4-Amino-m-Cresol, 5-Amino-4-Chloro-o-Cresol and 5-Amino-6-Chloro-o-Cresol, and lastly 4-Chloro-2-Aminophenol in an assessment that was published in 2004. In June of 2022, the Panel re-opened the safety assessment of these ingredients, since some hair dyes were banned for cosmetics in the European Commission. Because the Panel determined that the data on these amino cresol hair dye ingredients could not read across, rather than having one report they were broken out into separate reports.

This discussion pertains specifically to 6-Amino-m-Cresol, which has two reported uses in hair dyes and colors. The concentration of use provided by the Council in 2022, was at .69 percent in hair dyes and colors, down from 2.4 percent in previous report. And, of course, we've had some new evidence of genotox. Since June, no new data has been submitted.

Our motion is an insufficient data announcement. Our needs are method of manufacturing, dermal carcinogenicity studies, more in vitro genotox. We have micronuclei now. And, we can have a discussion afterwards about the allergy alert test, which this is a good time in this one to discuss later, after the motion.

DR. BERGFELD: Dr. Belsito?

DR. BELSITO: We wanted to hear what Tom had to say about the genotoxicity, so I'm presuming that's what you've delivered here. You want in vitro genotox studies.

DR. SLAGA: Yeah, I do.

DR. BELSITO: And you want a carcinogenicity study.

DR. COHEN: Tom, why don't you clarify your points and then we'll clarify our motion.

DR. SLAGA: With genotoxicity, you always have to go on weight of evidence because there's too many cases where, especially when you're dealing with in vitro, that sometimes it's positive but you may have also negative. And with this particular compound I think the weight of evidence is that it's not genotoxic.

DR. BELSITO: So then do we need that data? Do we need additional genotox data?

DR. COHEN: Tom, I thought we concluded we wanted more genotox data, but if you feel the micronucleus data that --

DR. SLAGA: Well, I would go with that, too. But I'm just telling you in general, you always go on the basis of the weight of evidence. And if we have more genotoxicity studies, I'm sure the weight of evidence we'll get from their studies will show that it's negative.

DR. BELSITO: Because in the original report, the genotox was all negative for this.

DR. ROSS: Correct.

DR. BELSITO: And now suddenly we're getting positives -- or a few positives. And it's very confusing.

DR. ROSS: I think the original 2004 conclusion was based on that negative genotox data.

DR. COHEN: Yes.

DR. SLAGA: Right.

DR. ROSS: It was a solid conclusion. And, actually, in the team meeting I said when I read this I just read the genotox data in the original report first. Then I went to the dossier and saw all this positive data. So I was really quite confused.

So I think the new data was the problem, and I think the Europeans used that as part of their decision to put this compound in Annex II, for cause, not for the lack of data.

And so, I mean, it wasn't just the in vitro data, if I remember our discussion -- looking at my team colleagues here. It wasn't just the in vitro data, there was some in vivo positive there, the in vivo micronuclei, for example, in mice. So we were a little concerned about that and felt that it would be useful to have more data to --

DR. BELSITO: Do you want more in vitro, or in vivo?

DR. ROSS: In vivo.

DR. BELSITO: But David said in vitro.

DR. COHEN: I misspoke. Thank you.

DR. BELSITO: I would agree with that.

DR. ROSS: Yeah.

DR. SLAGA: One more comment. In general, the Ames assay, it's fairly reproducible. One of the assays used is called clastogenic. It's really not mutagenic it's clastogenic. It's more related to tumor promotion, or increased cell proliferation. And there's more variability in that. And I put the weight on the Ames, the various testing strains it had to support for these particular type of compounds, which are not tumor promoters, they're a different type of compounds.

DR. BERGFELD: So, what are you really telling us, that we should ask for an Ames, or an in vivo study, or go with what we have?

DR. ROSS: I don't think you need more Ames. The Ames is actually consistent between the older data and the newer data.

DR. BERGFELD: Well, I'm just asking Tom what he's saying.

DR. ROSS: Okay.

DR. BERGFELD: Tom, did you hear me?

DR. SLAGA: Yeah.

DR. BELSITO: Do we need more Ames, more in vivo, or we don't need anything more?

DR. SNYDER: I don't think we need any more. But if the group would like to see more, I would prefer to see the Ames testing strains used. It would be more related to this type of compound.

DR. BERGFELD: Thank you.

DR. BELSITO: Right.

DR. BERGFELD: I think we have to have more discussion at the table here.

DR. COHEN: Yes.

DR. BELSITO: I think we wanted more in vivo, if you were going to get more data.

DR. COHEN: Yeah, that's right. That's where we come together. What about dermal carc?

DR. BELSITO: No, I think, dermal carcinogenesis is extremely expensive.

DR. SLAGA: It's only if you have positive genotoxicity.

DR. BELSITO: And, I think, no one's going to do that for a product that used in two.

DR. SLAGA: Right.

DR. BELSITO: Let's see what we get from additional in vivo.

DR. COHEN: Okay. I think that's --

DR. ROSS: I think the in vivo is important.

DR. BELSITO: Yeah.

DR. BERGFELD: Wait a minute. Tom has something to say and we talked over you, Tom. What did you say?

DR. SLAGA: Yeah, eliminate the dermal carcinogenicity.

DR. COHEN: Okay.

DR. BERGFELD: Okay. Thank you. So, Dr. Cohen, will you restate the conclusion and then the needs? And we'll vote on it.

DR. COHEN: Yes. So, our motion is an insufficient data announcement. Our needs are method of manufacturing and more in vivo genotoxicity.

DR. BERGFELD: And there's a second to that motion?

DR. BELSITO: Yes.

DR. BERGFELD: Now, I understand that Tom Gremillion has something to say. Tom?

DR. GREMILLION: I was going to ask about on vitro and in vivo, based on the European opinion, but it sounds like the panel is getting the data that's needed. I guess I'll just note for the record, that this product has been determine to not be safe for consumers at a concentration of 1.5 percent, based on this genotoxicity data. So, it seems prudent to gather more data on that. Thanks.

DR. BERGFELD: Thank you. We're going to call the question now, all those in favor of this conclusion with the needs assessment? Thank you, unanimous.

DR. COHEN: Wilma?

DR. BERGFELD: Go ahead, David.

DR. COHEN: Could we just talk about, perhaps in the use area, the allergy alert test.

DR. BERGFELD: Okay.

DR. COHEN: We didn't have time to talk about it yesterday. But in our description of it, we talk about it in a very generic way looking at it at 48 hours. And there's even a comment about the possibility of sensitization. This has always been a tricky issue. And there's some good peer-review data looking at allergy alert, because the instructions on the boxes are not uniform.

The FDA website describes applying a small amount on your skin and leaving it there for 48 hours.
A very good European study that looked at the instructions, about a third had no instructions on rinsing, just applying it. And probably another third or so said leave it on for 48 hours, and another third said leave it on for 45 minutes. There's no uniformity.

The FDA has 48 hours. The large companies have 48 hours, and other large companies have 45 minutes. There's another report that Toni Gaspary (phonetic) did, looking at 2-methoxymethyl-PPD, and their allergy alert test was 30 minutes.

I think we need to comment that, when we say allergy alert test, we're not talking about the same thing. There are all different things on different boxes. And we need that to unify.

DR. BELSITO: But isn't that the purview of the FDA, not ours?

DR. BERGFELD: Yes.

DR. COHEN: Well, we should at least comment on it in our Use section.

DR. BERGFELD: Suggested it go into our meetings that we alert the FDA that we're concerned about it. And possibly ask PCPC to help us get the word out; please harmonize this alert test.

DR. COHEN: Even within the same company, two different products can have different allergy alert tests.

DR. BELSITO: Um-hmm.

DR. COHEN: And I suppose, putting something on for 48 hours, every three or four weeks, is like sort of a slow HRIPT test over years. So, I don't know how long we need it on for.

DR. BERGFELD: Do we still have Dr. Manga from the FDA on board?

DR. MANGA: Yes, I'm still here. We will take this under advisement and take a look at it and give you a report back the next time.

DR. BERGFELD: Please. Put the mic in front of you, please.

DR. HELDRETH: And please state your name and affiliation.

MS. RALPH: My name is Mishka Ralph, Combe (phonetic) Incorporated. Cosmetic Europe is going to publish recommendations for the allergy alert test, based on the European study. And, I think, if I'm not mistaken, by the end of the year if not early next year.

DR. COHEN: That'd be great.

DR. BERGFELD: Thank you. All right, well, we have made our statement regarding the allergy alert test. We'll wait to hear from the FDA, and wait to see this publication.

DR. BELSITO: Great.

DR. BERGFELD: Thank you very much.

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 6-Amino-*m*-Cresol as Used in Cosmetics

Status:	Draft Tentative Amended Report for Panel Review
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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. This safety assessment was prepared by Christina Burnett, M.S., Senior Scientific Analyst/Writer, CIR.

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ABBREVIATIONS

ADME	absorption, distribution, metabolism, excretion
CIR	Cosmetic Ingredient Review
CMC	carboxymethylcellulose
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
DMSO	dimethyl sulfoxide
DNCB	dinitrochlorobenzene
EC_3	effective concentration inducing a stimulation index of 3
FDA	Food and Drug Administration
LLNA	local lymph node assay
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
SCCS	Scientific Committee on Consumer Safety
TG	test guideline
US	United States
UV	ultraviolet
VCRP	Voluntary Cosmetic Registration Program
wINCI; *Dictionary*	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)

DRAFT ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 6-Amino-*m*-Cresol, which is reported to function as a hair dye in cosmetic products. The Panel reviewed all relevant data and concluded that 6-Amino-*m*-Cresol…[to be determined.]

INTRODUCTION

6-Amino-*m*-Cresol, which according to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*) is reported to function in cosmetics as a hair colorant,[1] was previously reviewed by the Expert Panel for Cosmetic Ingredient Safety (Panel) as part of a safety assessment of six 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 6-Amino-*m*-Cresol… as used in oxidative and non-oxidative (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 due to 6-Amino-*m*-Cresol being banned for use in cosmetics by the European Commission.[3,4] 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 now be presented as individual stand-alone reports.

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 April 2023. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties. Excerpts from the summaries of the previous report on 6-Amino-*m*-Cresol are disseminated throughout the text of this re-review document, as appropriate, and are identified by *italicized text*. (This information is not included in the tables or the Summary section.)

CHEMISTRY

Definition and Structure

According to the *Dictionary*, 6-Amino-*m*-Cresol (CAS No. 2835-98-5) is the substituted aromatic compound that conforms to formula in Figure 1.[1] However, the use of regiochemical terms such as "*meta*-" (i.e., the "-*m*-" in 6-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 2-amino-5-methylphenol, is more common in the literature.

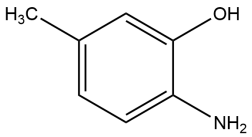

Figure 1. 6-Amino-*m*-Cresol

6-Amino-*m*-Cresol is used as a coupler in oxidative hair dye systems. Couplers, sometimes referred to as color modifiers, react with oxidized hair dye ingredients referred to as precursors. Couplers can react with 2 equivalents of precursor (to form a sort of trimer), or if "blocked," react with 1 equivalent of precursor (to form a sort of dimer). The methyl group on 6-Amino-*m*-Cresol, however, does not block coupling at either active site (i.e., neither *ortho*- nor *para*-); thus, this ingredient theoretically can react with precursors to form dimer- or trimer-like products (Figure 2).

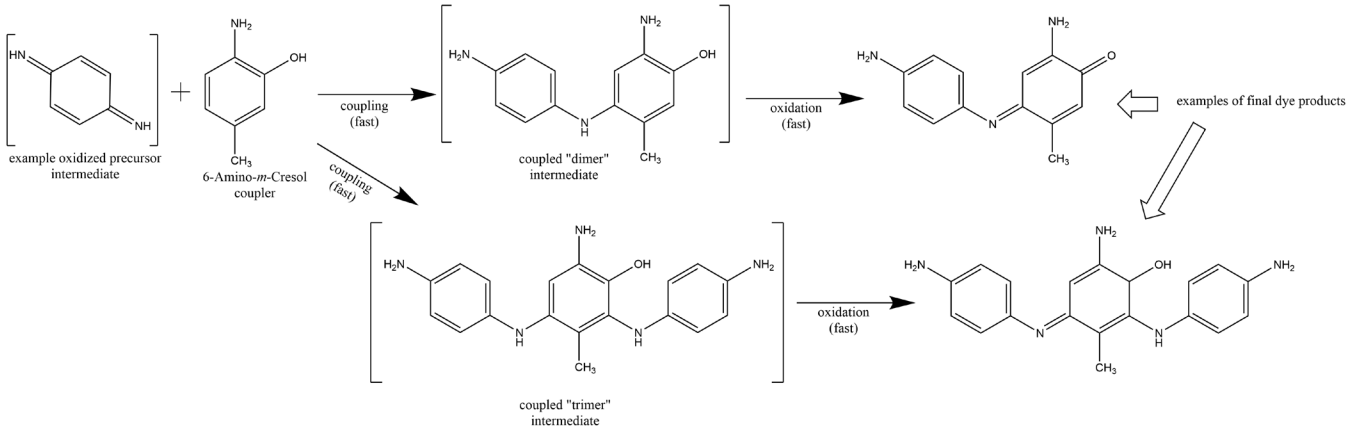

Figure 2. Examples of hair dye coupling with 6-Amino-*m*-Cresol

Chemical Properties

Chemical properties for 6-Amino-*m*-Cresol are summarized in Table 1. 6-Amino-*m*-Cresol (99.9% pure) has a molecular weight of 123.16 g/mol and is in the form of beige to reddish-brown crystals.[2] The estimated log P_{ow} is reported to be 1.14.[4]

Method of Manufacture

Method of manufacturing data were not included in the original report, were not found in the updated literature search, and unpublished data were not submitted.

Constituents/Impurities

Four different batches of 6-Amino-*m*-Cresol analyzed with nuclear magnetic resonance (NMR) were determined to be 98.9% - 99.7% (w/w) pure.[4] Water content ranged from 0.017% - 0.1% (w/w) and sulfated ash content ranged from < 0.01% - < 0.1% (w/w). Other constituents included 2-amino-4-methylphenol (< 1000 ppm, detection limit), 1,2-diamino-4-methylbenzene (< 20 - < 59 ppm, detection limit), and 4-methyl-2-nitroaniline (< 10 - < 23, detection limit).

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

According to 2023 VCRP survey data, 6-Amino-*m*-Cresol has no reported uses.[5] The results of the concentration of use survey provided by the Council in 2022 reported that this ingredient is used at 0.69% in hair dyes and colors, indicating use in at least 1 cosmetic formulation.[6] When the original safety assessment was published in 2004, 6-Amino-*m*-Cresol was reported to have 2 uses in hair dye and color formulations, according to 1998 VCRP data.[2] At that time, 6-Amino-*m*-Cresol was reported to be used at 2.4% in hair dyes and colors according to a survey performed by industry.

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. 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.[7,8] Hair dye products marketed and sold in the US, though, must follow the labeling requirements established by the Food, Drug, and Cosmetic Act.

Under European regulations for cosmetic ingredients, 6-Amino-*m*-Cresol is categorized in Annex II, the list of substances prohibited in cosmetic products in Europe.[3] The European Union's Scientific Committee on Consumer Safety (SCCS) determined that 6-Amino-*m*-Cresol could not be considered safe for hair dying purposes due to the genotoxic potential of this ingredient and its metabolite, *N*-acetyl-2-amino-5-methylphenol.[4] This determination was coupled with data that indicated that a dimer of the ingredient was formed under oxidative conditions and was found to absorb in human skin (in vitro). The SCCS assessors also objected to the INCI name, stating it was scientifically incorrect, and referred to this ingredient as 2-amino-5-methylphenol in the whole opinion.

TOXICOKINETIC STUDIES

Dermal Absorption

In Vitro

The dermal penetration potential of [^{14}C]6-Amino-*m*-Cresol (99% radiochemical purity) from a typical oxidative hair dye formulation was studied in viable human donor skin in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 428.[4] The formulation tested containing 0.75% [^{14}C]6-Amino-*m*-Cresol (with a reaction partner and hydrogen peroxide) was applied to the skin samples for 60 min. The area dosed was 100 mg/cm^2. Absorption was assessed by collecting receptor fluid (Dulbecco's minimum eagle medium) samples at 3 and 24 h post-dosing. At 24 h, the experiment was terminated by removing the skin samples from the well plates. The wells were rinsed with solvent and the skin was dried and the stratum corneum was removed by tape stripping. Exposed skin underwent an extraction procedure. Radioactivity was measured by liquid scintillation counting. The dermal delivery (amount of test material in the receptor fluid and in the skin) of the oxidative hair dye formulation containing 0.75% 6-Amino-*m*-Cresol was determined to be 0.34 % (2.77 µg/cm^2).

Animal

In an in vivo dermal absorption study, 6 male and 6 female PVG rats received [^{14}C]6-Amino-*m*-Cresol hemisulfate (95% radiochemical purity) in dimethyl sulfoxide (DMSO; 150 mg/ml; 0.1 ml/animal for 24 h) and in a formulation (15 mg/g; 1 g mixture/animal for 0.5 h) on the dorso-lumbar region.[4] The test sites were occluded (15 mg/animal; 1.667 mg/cm^2, 190 mCi). At the end of the dose periods, the plaster and foil were removed, and the animals were kept in individual metabolism cages to collect urine, feces, and expired air. The animals were killed 72 h post-doing and tissues were removed for radioactivity analysis. Approximately 14.25% of the applied dose of [^{14}C]6-Amino-*m*-Cresol dissolved in DMSO and 0.58% of the applied dose of the test material in a hair dye formulation were excreted, mainly in the urine. The remaining applied doses of the test material in DMSO (74.48%) and in formulation (82.78%) were recovered from the dressing, washing and application sites. No significant radioactivity levels were found in tissues 72 h after treatment.

Absorption, Distribution, Metabolism, Excretion (ADME)

In Vitro

The bioavailability of 6-Amino-*m*-Cresol (98.8% pure) across the intestinal barrier was investigated in human intestinal epithelial (TC-7) cells in vitro.[4] 6-Amino-*m*-Cresol (96 % recovery) revealed an apparent permeability coefficient (P_{app}) of 129.9 x 10^{-6} cm/sec and thus was classified to be of high permeability, indicating a complete absorption from the gastrointestinal tract. As the absorption from the gastrointestinal tract is likely to be permeability limited, the high permeability observed in this assay indicates a good absorption of 6-Amino-*m*-Cresol after oral administration.

The metabolism of 6-Amino-*m*-Cresol (98.8% pure) was studied in human, rat and mouse primary hepatocytes.[4] The test material (10µM) was incubated with the hepatocytes for 4 h. The metabolism of 6-Amino-*m*-Cresol was similar between the 3 hepatocyte types. The test material was extensively metabolized by sulfate and glucuronide conjugation. Although the human donors were phenotyped as rapid acetylators, no *N*-acetyl-2-amino-5-methylphenol could be detected.

In another metabolism study of [^{14}C]6-Amino-*m*-Cresol (97.9% - 98.9% radiochemical purity), human hepatocytes were suspended with 1, 10, 100, or 1000 µg/ml of the test material for 3 h or plated with 0.889, 8.89, or 88.9 µg/ml of the test material for 24 h.[4] The metabolic activity was assessed by measuring 7-hydroxycoumarin formation. [^{14}C]6-Amino-*m*-Cresol was readily metabolized and the profile of metabolites formed was concentration-dependent. Two metabolites of 6-Amino-*m*-Cresol, *O*-glucurono-2-amino-5-methylphenol and 2-amino-5-methylphenol-*O*-sulfate, were detected in studies with both suspended and plated hepatocytes. The substrate concentration dependency of formation of these metabolites was

similar in both studies. A third metabolite, *N*-acetyl-*O*-glucurono-2-amino-5-methylphenol, was detected in minor amounts only with plated hepatocytes, a test system that may more closely reflect the in vivo metabolic capability. In plated hepatocytes at the low-test concentration, the major metabolite was 2-amino-5-methylphenol-*O*-sulfate, while at higher substrate concentrations, *O*-glucuronidation became the predominant metabolic pathway. Metabolism was incomplete at the high concentrations both in suspended and plated human hepatocytes, suggesting saturation of phase II metabolism or enzyme inhibition.

The metabolism of an oxidative hair dye formulation containing 1.5% [^{14}C]6-Amino-*m*-Cresol (99% radiochemical purity) was investigated in viable human skin (thickness 580 - 650 µm) obtained from 3 female donors.[4] The skin samples (100 mg/cm^2) were exposed for 60 min to the test material. At 24 h post-dosing, the experiment was terminated by removing the skin samples. The samples of the receptor fluid (Dulbecco's minimum eagle medium) and skin extract were analyzed for metabolite profiling and identification. The metabolite profiling results indicate that *N*-acetylation is the major route of metabolism of 6-Amino-*m*-Cresol in skin. *N*-Acetyl-2-amino-5-methylphenol, 2-methyl-5-aminophenol-*O*-sulfate, and N-acetyl -2-amino-5-methylphenol-*O*-sulfate were identified as metabolites. A fourth metabolite (postulated to be *N*-acetyl-*O*-glucurono-2-amino-5-methylphenol) was detected in both receptor fluid and exposed skin samples. A dimer of 6-Amino-*m*-Cresol and a related substance were also identified in the receptor fluid and skin extract samples but were not quantified. The dimer was likely formed under the oxidative conditions of the formulation. The amount of *N*-acetylated metabolites was calculated to be 0.93 µg/cm^2; i.e., at least 34% of the total amount of 6-Amino-*m*-Cresol that was found in the receptor fluid or in the skin (2.77 µg/cm^2) was present in the form of these *N*-acetylated metabolites. However, the acetylator status of the skin samples of the 3 donors regarding arylamine *N*-acetyltransferase 1 (rapid or slow) is unknown. Thus, apart from other methodological restrictions, the evidence on the *N*-acetylation metabolic pathway in human skin is at best of semi-quantitative nature.

Animal

The ADME of [^{14}C]6-Amino-*m*-Cresol (97.9% radiochemical purity) was studied in Sprague-Dawley female rats after a single oral, intravenous, or dermal dose.[4] A total of 8 groups was used, with 4 groups used for a mass balance study and the remaining 4 groups used for toxicokinetics. Groups that received 25 or 400 mg/kg bw test material (in PEG 400) orally were comprised of 6 rats. Groups that received intravenous administrations of 25 mg/kg bw of the test material (in PEG 400/0.9% saline 40:60) were comprised of 4 rats. The groups that received 10 mg/kg bw test material (in acetone/ water 1:1) dermally on shaved skin (10 cm^2) were comprised of either 4 or 6 rats. In the mass balance groups, urine and feces were collected in intervals of 0 - 8, 8 - 24, 24 - 48, 48 - 72, and 72 - 96 h. Total radioactivity in urine, feces, tissues, and organs was determined. Selected urine and feces samples were pooled per group and the metabolite profile was investigated. In the toxicokinetics groups, blood was sampled alternatively from several rats at 0.25, 0.5, 1, 2, 4, 8, 24, and 48 h after dosing.

[^{14}C]6-Amino-*m*-Cresol administered orally was well absorbed, readily distributed, extensively metabolized and excreted mainly via urine. There is weak analytical evidence that metabolism resulted in oxidized and *N*-acetylated derivatives. After dermal application, 5.1% (0.019 mg/cm^2) of the radiolabeled dose was found in excretion, cage-wash, carcass and unexposed skin. This amount increased to 6.8% (0.026 mg/cm^2) when adding the residue in the exposed skin. Excretion took place mainly via urine but elimination was slower compared to oral administration. Intravenous results were not reported.[4]

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

The LD_{50} was calculated to be 1500 mg/kg in an acute study of male CD-1 mice dosed for 2 consecutive days with up to 1500 mg/kg 6-Amino-m-Cresol.[2] In a pre-experiment toxicity study, the maximum tolerated dose was 500 mg/kg in NMRI mice that received up to1500 mg/kg 6-Amino-m-Cresol in PEG 400. Mice that received 666 mg/kg 6-Amino-m-Cresol orally had tremor, anemia, and a slight to moderate reduction in activity within the first 2 h of being treated; however, no mortalities were observed. Two male rats that were orally dosed with 1200 mg/kg 6-Amino-m-Cresol in 1% carboxymethylcellulose had reduction of spontaneous activity, abdominal position, eyelid closure, and piloerection. In another experiment, the maximum tolerated dose was 1500 mg/kg in male rats that received either a single oral dose of 1500 or 2000 mg/kg 6-Amino-m-Cresol in 1% carboxymethylcellulose.

In an oral acute toxicity study, 6 male and 10 female Wistar rats received up to 1750 mg/kg bw 6-Amino-*m*-Cresol (purity not reported) via oral gavage, while 10 male and 10 female CF1 mice received up to 2000 mg/kg bw of the test material and 10 female CBL mice received up to 1250 mg/kg bw.[4] Mortality and clinical signs were checked daily during the 14-d observation period. All animals underwent gross necropsy after termination. Clinical signs of toxicity observed included sedation, tremor, accelerated respiration, and death. No macroscopic organ changes were noted. The LD_{50} were calculated as follows: 1375 mg/kg bw in male rats, 1225 mg/kg bw in female rats, 1020 mg/kg bw in male CF1 mice, 1225 mg/kg bw in female CF1 mice, and 750 mg/kg bw in CBL female mice.

Short-Term Toxicity Studies

Oral

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 wk.[2] The control group was dosed with 1 ml/100 g bw 0.5% carboxymethylcellulose (CMC). No significant observations occurred in the 50 mg/kg group. The 250 mg/kg group had increased activity 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 feed 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 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 Toxicity Studies

Oral

In a 90-d oral gavage study, 10 male and 10 female Wistar Bor: WISW/TNO (SPF) rats received a 10% suspension of 6-Amino-*m*-Cresol (98% pure) in 5% gum arabic at a dose of 800 mg/kg bw/d.[4] After week 6, the dose was reduced to 500 mg/kg bw/d due to clinical signs of toxicity and 2 animal deaths. Feed consumption, body weight, and body weight gains were significantly reduced in both sexes. Relative and absolute liver, kidney, and spleen weights were increased. No macroscopic or histopathological effects detected. An NOAEL could not be calculated.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Oral

Female Sprague-Dawley rats dosed orally with 5, 50, or 200 mg/kg 6-Amino-m-Cresol from days 6 to 15 of gestation had no mortalities attributed to treatment effects.[2] When compared to controls, no clinical changes or changes at necropsy were observed in any group. Body weight gain of all treated groups was comparable to the control group. No effect on pregnancy incidence was observed 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.

GENOTOXICITY STUDIES

In an Ames test using Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100, 6-Amino-m-Cresol (30 - 1000 µg/plate, with or without equal amounts of 6% hydrogen peroxide) was slightly mutagenic towards strain TA100 with and without metabolic activation.[2] It was not mutagenic towards the other strains. 6-Amino-m-Cresol (0.6 - 15.0 µg/ml in DMSO) was highly toxic to Saccharomyces cerevisiae diploid D7 cell cultures, with and without metabolic activation, but it did not induce increases in the frequency of convertant or aberrant colonies. 6-Amino-m-Cresol (12.5 - 200 µg/ml in DMSO) induced an increase in mutation to both ouabain and 6-thioguanine resistance in mouse lymphoma L5178Y cells with metabolic activation; however, the increase was not considered significant with or without metabolic activation. In a study using cultured male human peripheral lymphocytes, 6-Amino-m-Cresol hemisulfate (0.6 - 15.0 µg.ml in DMSO) did not significantly increase the number of aberrations as compared to controls, with or without metabolic activation.

In in vivo micronucleus tests in mice, 6-Amino-m-Cresol (up to 750 mg/kg) did not induce micronuclei in bone marrow cells.[2] 6-Amino-m-Cresol (3200 mg/kg in 4% gum Arabic) did not induce chromosome aberrations in Chinese hamster bone marrow cells. 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 hemisulfate did not cause sister chromatid exchanges in rat bone marrow chromosomes from a single oral dose of up to 600 mg/kg in distilled water. 6-Amino-m-Cresol did not induce unscheduled DNA synthesis assay at up to 1500 mg/kg. 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.

In vivo and in vitro genotoxicity studies for 6-Amino-*m*-Cresol are summarized in Table 2. 6-Amino-*m*-Cresol (0 - 5000 µg/plate in DMSO; 98.8% pure) was mutagenic in *S. typhimurium* strain TA100, with and without metabolic activation, but no mutagenicity was observed in strains TA98, TA102, TA1535, or TA1537.[4] Clastogenic effects were observed in a concentration-dependent and biologically relevant manner in a mouse lymphoma L5178Y $tk^{+/-}$ cell gene mutation test of

6-Amino-*m*-Cresol (97.8% pure) at up to 100 µg/ml with metabolic activation. A biologically relevant increase in mutant frequency was not observed at up to 160 µg/ml without metabolic activation. 6-Amino-*m*-Cresol (98.8% pure) induced an increase in human lymphocytes with micronuclei when tested at up to 26.8 µg/ml without metabolic activation, but no genotoxicity was observed when tested at up to 67.7 µg/ml with metabolic activation. In an alkaline Comet assay, a concentration-dependent and biologically relevant increase in the amount of DNA in the tail was observed when 6-Amino-*m*-Cresol (98.8% pure) was tested at up to 1232 µg/ml, with and without metabolic activation. In a rat micronucleus test, 6-Amino-*m*-Cresol (single intraperitoneal injection of up to 400 m/kg bw; purity not reported) induced a biologically relevant and dose-dependent increase in the number of bone marrow cells with micronuclei in both sexes.

CARCINOGENICITY STUDIES

Carcinogenicity data were not included in the original report, were not found in the updated literature search, and unpublished data were not submitted.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Dermal irritation and sensitization studies for 6-Amino-*m*-Cresol are summarized in Table 3. 6-Amino-*m*-Cresol (purity not reported) was not irritating in guinea pigs when tested at 1% in water and thickened with methylcellulose.[4] In a guinea pig sensitization test, no sensitization was observed to 3% 6-Amino-*m*-Cresol (purity not reported) in water and thickened with tylose. In a local lymph node assay (LLNA), 6-Amino-*m*-Cresol (98.9% pure) was a strong skin sensitizer when tested at up to 10% in DMSO (effective concentration inducing a stimulation index of 3 (EC_3) of 3.44%) and at up to 5% in acetone: water (1:1) mixed with olive oil (3:1) (EC_3 of 1.55%).

OCULAR IRRITATION STUDIES

In an ocular irritation study, 10 female Pirbright SPF guinea pigs had 1% 6-Amino-*m*-Cresol (aq.; purity not reported) instilled into 1 eye (0.1 ml).[4] No irritation was observed after the 24 h observation period. No further details provided.

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

SUMMARY

6-Amino-*m*-Cresol is reported to function in cosmetics as a hair colorant. 6-Amino-*m*-Cresol was previously reviewed by the Panel in a safety assessment that was published in 2004. At that time, the Panel concluded that 6-Amino-*m*-Cresol was safe as used in oxidative and non-oxidative (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 due to 6-Amino-*m*-Cresol being banned for use in cosmetics by the European Commission.

No uses were reported for 6-Amino-*m*-Cresol, according to 2023 VCRP data. The results of the concentration of use survey provided by the Council in 2022 report that this ingredient is used at 0.69% in hair dyes and colors, indicating use in at least 1 cosmetic formulation. When the original safety assessment was published in 2004, 6-Amino-*m*-Cresol was reported to have 2 uses in hair dye and color formulations, according to 1998 VCRP data. At that time, 6-Amino-*m*-Cresol was reported to be used at 2.4% in hair dyes and colors according to a survey performed by industry.

Under European regulations for cosmetic ingredients, 6-Amino-*m*-Cresol is categorized in Annex II, the list of substances prohibited in cosmetic products in Europe. The European Union's SCCS determined that 6-Amino-*m*-Cresol could not be considered safe for hair dying purposes due to the genotoxic potential of this ingredient and its metabolite, *N*-acetyl-2-amino-5-methylphenol. This determination was coupled with data that indicated that a dimer of the ingredient was formed under oxidative conditions and was found to absorb in human skin (in vitro).

In dermal penetration studies, the dermal delivery of an oxidative hair dye formulation containing a final concentration of 0.75% 6-Amino-*m*-Cresol was determined to be 0.34% in an in vitro study. In an in vivo study in rats, approximately 14.25% of the applied dose of 6-Amino-*m*-Cresol dissolved in DMSO (150 mg/ml) and 0.58% of the applied dose of a hair dye formulation containing 15 mg/g 6-Amino-*m*-Cresol were excreted, mainly in the urine. The remaining applied doses were recovered in the dressing washing, and application sites.

Toxicokinetics studies performed in vitro found 6-Amino-*m*-Cresol had the potential to be bioavailable after oral administration, and that metabolites may include *O*-glucurono-2-amino-5-methylphenol, 2-amino-5-methylphenol-*O*-sulfate, and *N*-acetyl-*O*-glucurono-2-amino-5-methylphenol. In rat ADME studies, 6-Amino-*m*-Cresol administered orally was well absorbed, readily distributed, extensively metabolized, and excreted mainly via urine. After dermal application, 5.1% of the radiolabeled dose was found in excretion, cage-wash, carcass, and unexposed skin. This amount increased to 6.8% when adding the residue in the exposed skin. Excretion was mainly in the urine, but elimination was slower compared to oral administration.

In an oral acute toxicity study in rats, the LD_{50} for 6-Amino-*m*-Cresol were calculated to be 1375 mg/kg bw in males and 1225 mg/kg bw in females. In mice, the LD_{50} were calculated to be 1020 mg/kg bw in male CF1 mice, 1225 mg/kg bw in female CF1 mice, and 750 mg/kg bw in CBL female mice.

An NOAEL could not be calculated in a 90-d oral gavage study in male and female rats that received a 10% suspension of 6-Amino-*m*-Cresol (98% pure) in 5% gum arabic at a dose of 800 mg/kg bw/d; after 6 wk, the dose was later reduced to 500 mg/kg bw/d due to clinical signs of toxicity and 2 animal deaths. Feed consumption, body weight, and body weight gains were significantly reduced in both sexes. Relative and absolute liver, kidney, and spleen weights were increased; however, no macroscopic or histopathological effects were observed.

6-Amino-*m*-Cresol (0-5000µg/plate in DMSO; 98.8% pure) was mutagenic in *S. typhimurium* strain TA100, with and without metabolic activation, but no mutagenicity was observed in strains TA98, TA102, TA1535, or TA1537. Clastogenic effects were observed in a concentration-dependent and biologically relevant manner in a mouse lymphoma L5178Y $tk^{+/-}$ cell gene mutation test of 6-Amino-*m*-Cresol (97.8% pure) at up to 100 µg/ml with metabolic activation. A biologically relevant increase in mutant frequency was not observed at up to 160 µg/ml without metabolic activation. 6-Amino-*m*-Cresol (98.8% pure) induced an increase in human lymphocytes with micronuclei when tested at up to 26.8 µg/ml without metabolic activation, but no genotoxicity was observed when tested at up to 67.7 µg/ml with metabolic activation. In an alkaline Comet assay, a concentration-dependent and biologically relevant increase in the amount of DNA in the tail was observed when 6-Amino-*m*-Cresol (98.8% pure) was tested at up to 1232 µg/ml, with and without metabolic activation. In a rat micronucleus test, 6-Amino-*m*-Cresol (single intraperitoneal injection of up to 400 m/kg bw; purity not reported) induced a biologically relevant and dose-dependent increase in the number of bone marrow cells with micronuclei in both sexes.

6-Amino-*m*-Cresol (purity not reported) was not irritating in guinea pigs when tested at 1% in water and thickened with methylcellulose. In a guinea pig sensitization test, no sensitization was observed to 3% 6-Amino-*m*-Cresol (purity not reported) in water and thickened with tylose. In an LLNA, 6-Amino-*m*-Cresol (98.9% pure) was a strong skin sensitizer when tested at up to 10% in DMSO (EC_3 of 3.44%) and at up to 5% in acetone:water (1:1) mixed with olive oil (3:1) (EC_3 of 1.55%). No ocular irritation was observed in guinea pigs to 1% 6-Amino-*m*-Cresol (aq.; purity not reported).

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.

Carcinogenicity studies on 6-Amino-*m*-Cresol were not included in the original report, were not found in the updated literature search, and unpublished data were not submitted.

DRAFT DISCUSSION

[Note: This Discussion is in the draft form, and changes will be made following the Panel meeting.]

In accordance with its Procedures, the Panel evaluates the conclusions of previously-issued reports approximately every 15 years. In 2004, the Panel published a final report on 6-Amino-*m*-Cresol and concluded that this ingredient was safe for use in oxidative and non-oxidative (semi-permanent) hair dyes. This report has been reopened due to 6-Amino-*m*-Cresol being banned for use in cosmetics by the European Commission. In this amended report, the Panel concluded that the available data are… [to be determined].

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 Food, Drug, and Cosmetic Act (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.

CONCLUSION

To be determined.

TABLES

Table 1. Chemical properties of 6-Amino-*m*-Cresol

Property	Value	Reference
Physical Form	Beige to reddish-brown crystals	[2]
Molecular Weight (g/mol)	123.16	[2]
Density (g/ml @ 20 ºC)	0.77	[4]
Vapor pressure (mmHg @ 25 ºC)	0.00308 (estimated)	[4]
Sublimation Point (ºC)	156-159 (skips melting at 1 atm)	[4]
Water Solubility (g/l @ 20 ºC & pH 7.65)	5.9	[4]
Acetonitrile Solubility (g/l)	34	[4]
DMSO Solubility (g/l)	> 100	[4]
Acetone/Water Solubility (1:1; g/l)	33	[4]
log P_{ow}	1.14 (estimated)	[4]
UV Absorption (wavelength (λ)) (nm) in ethanol	210, 235, and 291	[2]

Table 2. Genotoxicity studies of 6-Amino-*m*-Cresol

Concentration/Dose	Vehicle	Test System	Procedure	Results	Reference
			IN VITRO		
0, 100, 316, 1000, 2500, or 5000 µg/plate; 98.8% pure	DMSO	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537	Ames test in accordance with OECD TG 471; with and without metabolic activation	Mutagenic in TA100, with and without metabolic activation; evidence of toxicity observed at and above 2500 µg/plate in all strains except for TA102, which started at 1000 µg/plate	[4]
0.1 - 160 µg/ml without metabolic activation; 0.5-100 µg/ml with metabolic activation; 97.8% pure	culture medium	mouse lymphoma L5178Y $tk^{+/-}$ cells	Mammalian cell gene mutation test in accordance with OECD TG 476; with and without metabolic activation	Clastogenic; concentration-dependent and biologically relevant increase in mutant frequency observed with metabolic activation, an increased occurrence of small colonies was found indicating a mutagenic or clastogenic effect; without metabolic activation, a biologically relevant increase in mutant frequency was not observed	[4]
8.6 - 26.8 µg/ml without metabolic activation; 25.0-67.7 µg/ml with metabolic activation; 98.8% pure	DMSO	human lymphocytes	Micronucleus test in accordance with OECD TG 487; with and without metabolic activation	Genotoxic; test material induced an increase in lymphocytes with micronuclei without metabolic activation; no biologically relevant increases in micronuclei observed with metabolic activation	[4]
25 – 1232 µg/ml without metabolic activation; 308-1232 µg/ml with metabolic activation; 98.8% pure	DMSO	V79 cells	Alkaline Comet assay; with and without metabolic activation	Genotoxic; concentration-dependent and biologically relevant increase in the amount of DNA in the tail observed with and without metabolic activation	[4]
			IN VIVO		
0, 100, 200, or 400 mg/kg bw; purity not reported	2.5% hydroxypropylcellulose	Groups of 5 male and 5 female Crl: CD (SD)BR rats	Mammalian erythrocytes micronucleus test in accordance with OECD TG 474; single intraperitoneal injection	Genotoxic; test material induced a biologically relevant and dose-dependent increase in the number of bone marrow cells with micronuclei in both sexes	[4]

Table 3. Dermal irritation and sensitization studies of 6-Amino-*m*-Cresol

Concentration/Dose	Vehicle	Test Population	Procedure	Results	Reference
IRRITATION					
ANIMAL					
1% test material (purity not reported)	water, thickened with methylcellulose	10 female albino SPF guinea pigs	Dermal irritation study; test material applied on abraded skin on area of 3 cm x 4 cm; 3 times daily for 20 min for 2 d (consecutive)	Negligible erythema observed on day 1 that was not recognizable on day 2; no edema or crusts observed	[4]
SENSITIZATION					
ANIMAL					
3%; purity not reported	water, thickened with 0.5% tylose	15 female albino Pirbright SPF guinea pigs with additional 10 as controls	Magnusson and Kligman guinea pig sensitization study; test material applied to abraded flanks without occlusion; 5 d/wk for 3 wk	No erythema or edema up to 72 h after challenge	[4]
0.5, 1.5, 5, or 10%; test material 98.9% pure	DMSO or acetone: water (1:1) with olive oil (3:1)	Groups of 5 CBA/J female mice	LLNA in accordance with OECD TG 429; tested with 2 different vehicles: 6-Amino-*m*-Cresol tested with DMSO at up to 10%; with the acetone: water:olive oil mix at up to 5%; positive control was 1% *p*-phenylenediamine in DMSO	Strong skin sensitizer; EC_3 value in DMSO was 3.44%; EC_3 in acetone:water:olive oil mix was 1.55%	[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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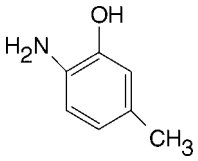
used in hair tints and hair dyes and colors at concentrations of ≤1%, ≤5%, and ≤1%, respectively.

CHEMISTRY

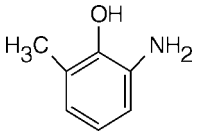
Definition and Structure

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



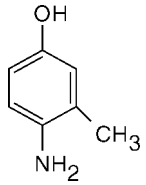
6-Amino-*m*-Cresol is also known as 4-Amino-3-Hydroxytoluene; 2-Amino-5-Methylphenol; Phenol, 2-Amino-5-Methyl-; 2-Hydroxy-4-Methylaniline (Pepe, Wenninger, and McEwen 2002); *m*-Cresol, 6-Amino; 6-Amino-3-Cresol; 6-Amino-3-Methylphenol; 2-Hydroxy-*p*-Toluidine; 5-Methyl-2-Aminophenol (Regulated Chemicals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



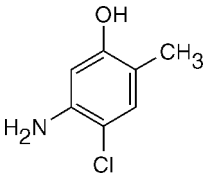
6-Amino-*o*-Cresol is also known as 3-Amino-2-Hydroxytoluene; 2-Amino-6-Methylphenol; Phenol, 2-Amino-6-Methyl-; 6-Amino-2-Methylphenol; Phenol, 6-Amino-2-Methyl-; 2-Hydroxy-3-Methylaniline (Pepe, Wenninger, and McEwen 2002); *o*-Cresol, 6-Amino; 6-Methyl-2-Aminophenol (Regulated Chemicals Listing 1998);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



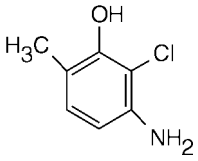
4-Amino-*m*-Cresol is also known as 2-Amino-5-Hydroxytoluene; 4-Amino-3-Methylphenol; Phenol, 4-Amino-3-Methyl-; 4-Hydroxy-*o*-Toluidine (Pepe, Wenninger, and McEwen 2002); 3-Methyl-4-Aminophenol (James Robinson Ltd. 1998); *p*-Amino-*m*-Cresol; *m*-Cresol, 4-Amino-; 4-Hydroxy-2-Methylaniline; *p*-Hydroxy-*o*-Toluidine;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



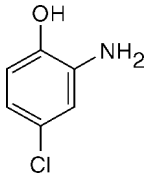
5-Amino-4-Chloro-*o*-Cresol is also known as 5-Amino-4-Chloro-2-Methylphenol; Phenol, 5-Amino-4-Chloro-2-Methyl- (Pepe, Wenninger, and McEwen 2002); and 2-Methyl-4-Chlor-5-Aminophenol (Henkel KGaA 1994).

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:

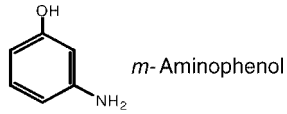


4-Chloro-2-Aminophenol is also known as 2-Amino-4-Chlorophenol; Phenol, 2-Amino-4-Chloro-; 2-Hydroxy-5-Chloroaniline; CI 76525 (Pepe, Wenninger, and McEwen 2002; Regulated Chemicals Listing 1998); 5-Chloro-2-Hydroxyaniline; *o*-Amino-*p*-Chlorophenol; *p*-Chloro-*o*-Aminophenol; and C.I. Oxidation Base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

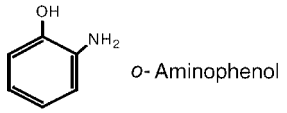
TABLE 1
Physical and chemical properties of 5-Amino-4-Chloro-*o*-Cresol and 5-Amino-6-Chloro-*o*-Cresol (Henkel KGaA 1994, 1996)

	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)

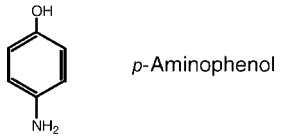
Structure of Related Ingredients



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

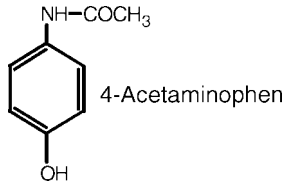
No data were available on ingredients in this safety assessment, but data on related ingredients are summarized below.

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.

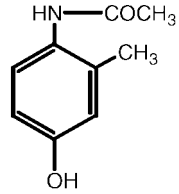
Acetaminophen

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).

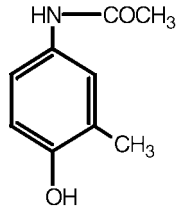


4-Acetaminophen

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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