BLUE

Anisoles

CIR EXPERT PANEL MEETING

DECEMBER 12-13, 2011

Cosmetic Ingredient Review
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Memorandum

To: CIR Expert Panel Members and Liaisons

From: Christina L. Burnett

Scientific Writer/Analyst

Date: October 31, 2011

Subject: Draft Final Report on 2-Amino-4-Hydroxyethylaminoanisole and its Sulfate Salt

At the September 2011 Panel Meeting, the CIR Expert Panel issued a Tentative Report for the hair dye ingredients, 2-Amino-4-Hydroxyethylaminoanisole and 2-Amino-4-Hydroxyethylaminoanisole Sulfate, with the conclusion "…safe for use in hair dye formulations. Were the free base to be used in the future, the expectation is that it would be used at concentrations similar to the sulfate salt. The Expert Panel cautions that these ingredients should not be used in products in which *N*-nitroso compounds are formed."

Since the September meeting, no new data have been found or received. If the Panel is satisfied with the discussion and conclusion, a Final Report should be issued.

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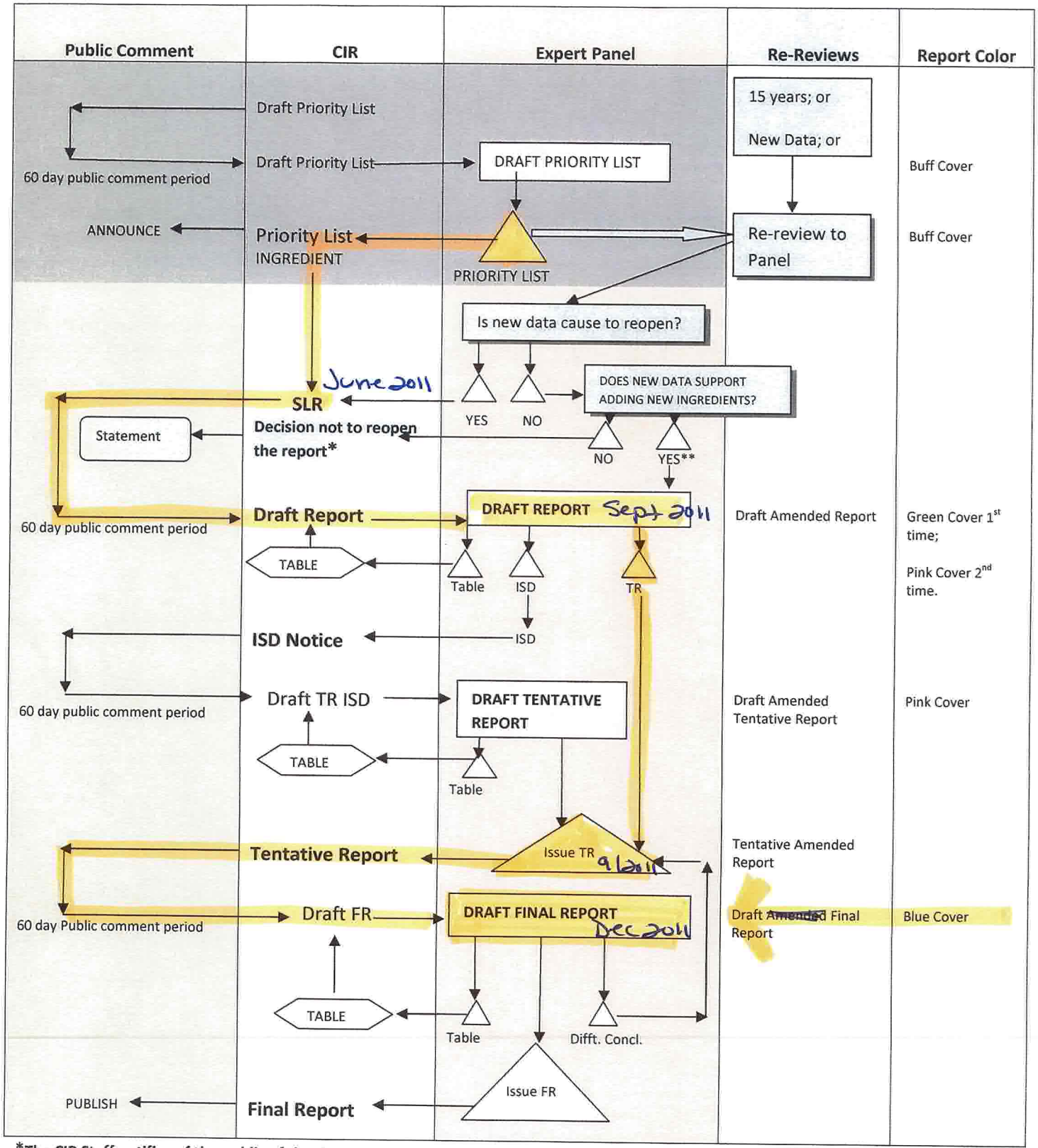
2-Amino-4-Hydroxyethylaminoanisole

Dec 2011

SAFETY ASSESSMENT FLOW CHART



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

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

△ Expert Panel Decision

▭ Document for Panel Review

⇨ Option for Re-review

2-Amino-4-Hydroxyethylaminoanisole History

June 2011

CIR issued a Scientific Literature Review.

September 2011

A tentative safety assessment with a conclusion of safe as hair dye ingredients in the present practices of use and concentration was issued for 2-amino-4-hydroxyethylaminoanisole and 2-amino-4-hydroxyethylaminoanisole sulfate, except that these ingredients should not be used in hair dye products in which *N*-nitroso compounds can be formed. The CIR Expert Panel reviewed the data that were largely provided by the Personal Care Products Council's Hair Coloring Technical Committee on 2-amino-4-hydroxyethylaminoanisole sulfate. The Panel noted that the sulfate salt has 94 uses in hair dye products at concentrations up to 1.5% after dilution, but that no uses or use concentrations were reported for the parent compound.

2-Amino-4-Hydroxyethylaminoanisole Family Data Profile* – December 2011 – Writer, Christina Burnett														
	Reported Use	log P value	Toxicokinetics Data	Animal Tox – Acute, Dermal	Animal Tox – Acute, Oral	Animal Tox, Acute, Inhalation	Animal Tox – Rptd Dose, Dermal	Animal Tox, Rptd Dose, Oral	Animal Tox – Rptd Dose, Inhalation	Repro/Dev Tox**	Genotoxicity	Carcinogenicity - Dermal	Dermal Irr/Sens	Mucosal Irritation
2-Amino-4-Hydroxyethylaminoanisole	X	X												
2-Amino-4-Hydroxyethylaminoanisole Sulfate	X	X	X		X		X	X		X	X		X	X

*"X" indicates that data were available in a category for the ingredient

SEARCH STRATEGY FOR 2-AMINO-4-HYDROXYETHYLAMINOANISOLE AND ITS SULFATE SALT (Performed by Christina Burnett)

April 27, 2011: SCIFINDER search for CAS No. 83763-48-8

- Initial search with no limits resulted in 74 references.
- Limited search to books, clinical trials, commentary, conference, editorial, historical, journals, preprints, reports, and reviews; 5 references came back.

May 2, 2011 SCIFINDER search for CAS No. 83763-47-7

- Initial search with no limits resulted in 326 references.
- Limited search to books, clinical trials, commentary, conference, editorial, historical, journals, preprints, reports, and reviews; 3 references came back.
-

Search Terms	TOXLINE	PUBMED	EU
April 27, 2011			
2-Amino-4-Hydroxyethylaminoanisole Sulfate	0	0	Max concentration after mixing should not exceed 1.5%. Do not use w/ nitrosating agents. Max nitrosamine content is 50µg/kg.
83763-48-8	0	0	
May 2, 2011			
2-Amino-4-Hydroxyethylaminoanisole	0	0	Max concentration after mixing should not exceed 1.5% (as sulfate). Do not use w/ nitrosating agents. Max nitrosamine content is 50µg/kg.
83763-47-7	0	0	
aminohydroxyethlaminoanisole	0	0	

Total references ordered: 3

Search updated October 26, 2011.

Minutes

Dr. Marks' Team Minutes – September 26, 2011

DR. MARKS: So next we have anisole, the 2-Amino-4- Hydroxyethylaminoanisol and its Sulfate Salts. This is the first time we've seen this report. It's a hair dye. There is zero uses of the lead compound I saw in 94 uses of the sulfate salts and the maximum concentration is three percent prior to dilution. I'll open it up for discussion. Ron, Tom, and Ron, I particularly -- Tom, why don't you address the nitrosamine mentioned on page 7 of book? And do we have any add-ons that we want to --

DR. SHANK: I don't know about add-ons. This is an oxidated hair dye. We have sufficient data to say I think it's safe for that use, both the sulfate and the free base.

DR. SLAGA: I agree, safe as used.

DR. SHANK: The nitrosation would be the boilerplate, whatever we decide that's going to be.

DR. MARKS: So Tom, fine. And Ron Hill?

DR. HILL: When I look through this, and up until I'm sort of reviewing my notes, I kind of come to the same conclusion but I'm noticing that we don't have any carcinogenicity study. The structure of this is such that I would have liked to have seen that data. I need to sort of have a sense for how much suggestion from the muta -- excuse me, the genotoxicity data we would have concern for that because what I'm noticing is that I don't know anything about the vehicle when they've done these toxicity studies in the hair dye formulations because I don't have information about the pH which would be all important in terms of any dermal absorption, but I notice a lot of the dermal tox and the dermal absorption studies were done with either the dihydrochloride or the sulfate in tap water, which means we're not going to get a good picture of how much free base is present, which would be the one that would be penetrating any skin layers. So it almost seems to me that there's an artificial skewing of the data towards safety if we, in fact, don't know the key piece of information, which is how much freebase is actually available in these dermal studies. And that should be zero if you take the sulfate or the dihydrochloride and dissolve it in tap water when you do that work. Or nearly zero.

DR. SLAGA: There's sufficient genotoxicity data even though some of the mammalian is plus or minus. Most of the essential (inaudible) in vivo mammalian data is negative, as well as the --

DR. HILL: Okay. So I need some tox consult here. When it says the compound was mutagenic and that a mouse lymphoma assay -- I can ignore that?

DR. SLAGA: Well, by ignoring it --

DR. HILL: High concentrations.

DR. SLAGA: -- in some cases it's positive and in some cases it's negative.

DR. HILL: Okay.

DR. SLAGA: But if you take the total mammalian genotoxicity, it's more negative than positive. That happens on a lot of mammalian (inaudible). In a lot of cases it's more clastogenic instead of truly genotoxicity.

DR. SHANK: Gene mutation assays were done on the ingredient itself but the way it's used, it's used in an oxidative hair dye and most of that ingredient dissipates while it's still on the hair.

DR. HILL: So it's being converted -- it's being converted to something else.

DR. SHANK: Right. So the actual exposure to the individual --

DR. HILL: And that was my gut response when I first read through this.

DR. SHANK: Okay.

DR. MARKS: Any other comments? So the discussant points but the bottom-line is that we would support a motion that a draft tentative report be issued on these two compounds as safe for use as a hair dye. Is that correct?

DR. SLAGA: Yes.

DR. HILL: Let me ask a question.

DR. SHANK: As an oxidative hair dye.

DR. SLAGA: Yeah.

DR. HILL: Let me ask the question then because I guess I was thinking about it that way but then I didn't go back and re-read until now. On page -- the first page of the report, which is Book page 7, we have then a structure of recorded dye but we don't have any studies done on that to know do we have a problem biologically with the activity of that dye? Is that dye getting formed only in hair, never having exposure to the scalp? Because we're talking about 1.5 percent. That's a nontrivial concentration (inaudible). And I'm looking at the structure of that dye and thinking, yeah, there could be some biological activities there and that's not been studied best I can tell. We're only going by the studies of the parent compound and again, in many cases that's done with dihydrochloride with the sulfate. We're not looking at an absorbable species if it's in tap water and we don't have information at all

about -- or at least I don't have information from what I have here about what that formulation is actually like when they do the studies in the hair formulation. I don't know what the pH is. I don't know if they were co-solvents, any of that. The question is if we have the dye and it's there at 1.5 percent, if we assume complete conversion, which is kind of what the assumption is here, what will be the consequence of having that dye under the conditions of use? Will it penetrate the skin? Will it be absorbed? Will it have any of the biological consequences that we're trying to avoid here? I don't have any information to know.

But looking at the structure, that surely should be absorbable. That surely could have potential for biological activity looking at the structure. I mean, there are structure alerts in that dye structure from where I sit and we don't have any information on that.

DR. MARKS: Julie.

SPEAKER: I just wanted to make sure the panel was aware that the reaction products, the oxidative hair dyes, have been the topic of a considerable body of work done by the industry to submit in the European Union to the SCCS and they have issued an opinion on the reaction products this last year. And so this hasn't been brought to the attention of this panel because you have focused only on the ingredient rather than the reaction product, which is technically not an ingredient but if you chose to, you could invoke that reaction product's opinion to address the questions that Ron is raising here.

What we've discovered is that we've done dermal penetration studies on a series of reaction products that were intended to cover the span of molecular weights and log Kws for the large number of reaction products that can be formed from oxidative hair dyes. And what we've discovered is that the dermal penetration of these is substantially lower than the parent than the dye itself, the pre-cursor. So in reality I think the key thing still remains the safety assessment of the ingredient rather than the reaction.

DR. HILL: Well, I totally disagree with the last thing that you said because if we want to know is it safe to a human being, then we need to know whatever products are formed. And I'm looking at the structure on Book page 1 and saying this should be imminently absorbable. I don't see anything that suggests the log p would be -- would render it unabsorbable. The molecular weight is nice and small. I don't see in this particular case with this particular purported dye, and also I sketched some possible cyclization products that might form from that purported dye. All of those would be absorbable, and I think we need to know something about that to make a

conclusion.

So I didn't have access to the review that you're talking about, nor was it referenced in here. I can't say I didn't have access because obviously it's got to be publicly available. I didn't -- I wasn't -- it wasn't brought to my attention and I'm sure I should have noticed this three weeks ago right before Labor Day when I first got this book, but the fact of the matter is I'm just noting this now.

DR. MARKS: So Julie, yeah, could you answer why it's not absorbed and --

SPEAKER: Yes.

DR. MARKS: -- perhaps I would say this should be included in the discussion and then referenced. But what you're saying is reassuring.

SPEAKER: Yeah, let me just answer Ron's question.

The basis for concluding that the absorption is low of the reaction products versus the precursors or couplers was based on empirical experimental data and under conditions of use in the presence of hair and the amount of reaction product that actually forms is relatively small so that factors into the amount that could potentially be absorbed then if the amount formed is small. So rather than from a theoretical point of view, from an actual experimental point of view under simulated use conditions, the exposure is small.

DR. HILL: Okay, we have a circular argument here because we're saying up to 1.5 percent in the formulation. So either we've got somewhere near 1.5 percent of the parent compound before it reacts or we're saying, well, most of it's converted under conditions of use to a purported dye, which as I say, I'm looking at this structure and a couple of things I could see potentially could happen to it and thinking we have a very absorbable molecule in this particular case for this particular oxidative ingredient. Actually, this is not an oxidative ingredient. There's nothing oxidative about that 2-amino- 4-hydroxyethylaminoanisol. It's not oxidative but it is reacting in a way that's being oxidized with this iminium. So, but once it reacts with the iminium, we have a very absorbable molecule.

DR. MARKS: Julie, is that published?

SPEAKER: The opinion is published. It's on the website.

DR. MARKS: Okay, so --

DR. HILL: I want to know about this specific ingredient, not just, you know, in general these

kinds of ingredients in reaction products. I want to know how much of this particular dye forms and can be absorbed because --

DR. MARKS: Ron Shank.

DR. SHANK: In the chemical reaction you're looking at there's one molecule missing and that's hydrogen peroxide.

DR. HILL: Isn't that what makes the imminium? Hydrogen peroxide is not going to react with that anisole.

DR. SHANK: Well, my understanding is the chemical reaction takes place in the hair -- the protein, etcetera -- and becomes part of the hair. That's why it's a permanent hair dye. When hair is shampooed, the dye is still part of the hair. It does not come out.

DR. HILL: Okay. Grant you. But you put it on, I mean, it's all over the scalp as well as the hair. Right? So there's no thing --

DR. SHANK: You don't put it on like a shampoo.

DR. HILL: I know that. I've seen my wife dye her hair. But there's plenty of scalp exposure, is there not?

DR. SHANK: There is not. If it's done right it's not.

SPEAKER: There shouldn't be.

DR. HILL: You do it like this and I guess what I'm saying is how do you preclude scalp exposure?

DR. SHANK: We've gone over this.

DR. HILL: I know, but I haven't been on the panel when you've gone over this 100 times so educate me. I need to be educated here.

DR. MARKS: I'll tell you what, let's -- hmm. Let's do the education. We'll move on the next and I'll let the two Rons discuss offline and then we can bring it back up tomorrow if that sounds good.

Julie, I think it's -- actually, Christina, I'm going to put it on you to get that. I think that's helpful information on reactive products and should be in the discussion along with obviously that we have the hair dye epidemiology.

DR. HILL: I have been on long enough to see the last round of epidemiology.

DR. MARKS: Yeah, exactly. I know.

DR. HILL: I appreciate its usefulness and its limitations.

DR. MARKS: Yeah. Julie.

SPEAKER: Yeah, I just wanted to also add that, you know, the last time we presented on the epidemiology I had a section of the presentation I had to eliminate because we had that new study that we needed to spend time on, but I would offer that in the future I could give the panel a presentation on oxidative hair dyes. So everyone, the new members, can also.

DR. HILL: Yeah. I was here for the epidemiology. It was great and we had a large study from Japan, I think, that was particularly enlightening. I don't know if you talked about it.

SPEAKER: There was the New England study.

DR. HILL: Or was it China? It was -- I think it was China because it was a huge number of individuals involved and it was s--

SPEAKER: Oh, that was the prospective cohort -- the cohort study.

DR. HILL: Yeah, and it was great and valuable. And with that, along with your presentation, I had 8 --

DR. MARKS: So, Ron, Tom, and Ron, would you like Part 2 of Julie's presentation on the reactive products?

DR. SHANK: I think it would be good for the panel, especially the new members of the panel to hear.

DR. MARKS: Yeah.

DR. SHANK: Hear that because it was not intuitively obvious to me until I heard the presentation from the hair chemists that what's actually going on.

DR. HILL: Because I see exactly how that applied with hydroquinone. I mean, and there have been several other ingredients that have gone by that I have also had no problem with we've discussed and I've thought about numerous of these things. This is the first one where I feel less comfortable.

DR. MARKS: Monice.

MS. FIUME: I just want the panel to be aware we have been trying to fit it in but as you are well aware with the workload that the panel has had the last few meetings, it's the timing of trying to fit it in because have talked about --

DR. MARKS: Good.

MS. FIUME: -- the chemistry of hair dyes. So we are trying to fit it in.

DR. MARKS: So you've wetted our appetite and we're looking for the main course here in the future.

MS. FIUME: I'll try to get it in really soon.

DR. MARKS: Well, I'm not sure of the urgency. I'm reassured by what we've heard and we'll be able to get the actual reference to review also about that.

Okay. So even though I'm not the one who's going to be moving this, although they may have a different opinion with the Belsito team, I'll move that we issue a draft tentative report that these two hair dye ingredients are safe as used.

Do you want to put in the conclusion as an oxidative hair dye or you wouldn't put that in, Ron Shank, would you, just to safe as used?

DR. SHANK: Safe as used.

DR. MARKS: Yes. And the discussion will entail, as I mentioned earlier we do with all these hair dyes, the epidemiology boilerplate and link to our website and also we'll have some discussion about the reactive products.

Anything else?

DR. BERGFELD: Could I ask a technical question? Usually in the discussion you don't reference something. So if you're going to reference this particular piece it should go somewhere in a paragraph before the summary.

DR. BELSITO: Okay. So, the amino anisole, this is a hair dye. Okay, it's the first time we're look at it. Literature survey was done, technical comments. Updates to the epidemiology are online. I mean, I thought it was fine, safe as used in a hair dye product.

SPEAKER: (inaudible)

DR. BELSITO: Right, I mean, the usual, functionary statements.

DR. ANSELL: Yes.

DR. BELSITO: I mean, but even then, surprisingly, compared to PPT, it's really not a sensitized there. So, if we're going to let PPT out there, why are we going ban this stuff?

DR. ANDERSEN: I think the question though, and maybe Jay can frame it better than I can, is what's the right way to say don't form nitrose agents? On page 1, Panel Book page 7, our language says, "Should be formulated to avoid the formulation of nitrosamines." And I can't remember whether that's the language that the council likes or not like.

DR. ANSELL: Well, it's some language, although not in this report, which suggests that people are unaware of this and we did not like that, but, for some reason, it didn't pop up in this discussion.

DR. BELSITO: In the discussion, what we were using before was when they would be formed or something implying that --

DR. ANDERSEN: It's implying that it'd have it automatically.

DR. ANSELL: Right.

DR. BELSITO: But that there would be formation and should not be used where nitroso compounds will be formed or something, and that's --

DR. ANSELL: Monice, can you jump in?

SPEAKER: But what's the --

SPEAKER: We don't use them.

DR. BELSITO: Right.

SPEAKER: Yes.

DR. ANDERSEN: What was the council's suggested language for the DEA amides, et cetera?

MS. FIUME: "Products containing these ingredients should be formulated to avoid the formation of nitrosamines."

DR. ANDERSEN: Sounds very close to the way that Christina raised it.

MS. FIUME: Actually, I think (inaudible).

DR. KLAASSEN: To avoid or to minimize?

DR. ANSELL: Okay, the language we objected to was the panel cautions that, "These ingredients should not be used in cosmetic products in which N-nitroso compounds are formed." And we don't like the idea that N-nitroso compounds are formed because we don't sell anything with N-nitroso compounds. And, so, and then Carol struck up some language which, on first reading, I can't tell the difference between this and that either.

DR. BELSITO: Right.

DR. LIEBLER: I thought the new language was acceptable.

DR. ANDERSEN: "The Expert Panel cautions that products containing these ingredients should be formulated to avoid the formation of nitrosamines," and that certainly works for me.

DR. SNYDER: Safe as used.

DR. BELSITO: Safe as used.

DR. LIEBLER: I have some editorial comments on file.

DR. BELSITO: Okay, anything other than editorial comments that Christine can pick up?

DR. SNYDER: So, the nitrosamine (inaudible) goes in as a part of the conclusion?

DR. BELSITO: No.

DR. SNYDER: It doesn't go with "when formulated to?"

DR. BELSITO: In the discussion, no.

MS. BURNETT: It goes in the discussion (inaudible).

DR. SNYDER: Okay, thank you.

Full Panel Meeting – September 27, 2011

DR. BELSITO: This is the first time that we're looking at this report. It is a hair dye so subject to all the usual issues with hair dyes that as long as it's labeled to be tested, the sensitization and irritation issues aren't there. Having looked at this, safe as used when formulated to avoid the formation of N-nitroso compounds.

DR. BERGFELD: Motion?

DR. MARKS: Second.

DR. BERGFELD: Second. Is there any discussion? I'll call for the vote. All those in favor? Ron's got his hand up early. Let's go for it. Are there any discussant points that have to go in or comments that need to be made or editorials? Seeing none, we'll move on.

DR. MARKS: Obviously hair epidemiology. I'm not sure that Don mentioned that. The other thing that came in our team meeting which I think we need to address are reactive products so that in the discussion we should talk about and there was a reference to a paper that already exists on that.

DR. HILL: What I was bothered by was that if you look at the structure of the dye substance that's proposed to be formed, there doesn't seem in this case to be anything that would prevent dermal absorption. In a lot of cases we'll see the dyes and there will be more than charge or charge groups. This is small molecular weight, nothing that's sufficiently basic or acidic to suggest that it would be highly charged and I can't see any reason why it wouldn't get into the scalp. Julie Skare was with us yesterday and she made comment that it was formed in a small amount and if only a small percentage gets converted to that dye, that means the rest of it is available for absorption so then I had a question about the toxicology of the parent substance. We got into a sort of circular argument about, yes, it's supplied as a hair dye but it's being reacted and heavily converted to a dye substance and I said the dye could get in but we have no toxicology on that dye substance. Then the statement was made only a small percentage of it gets converted to the dye substance and that would mean we have a high amount of the unreacted parent substance and we don't have I think much in the way of toxicology on that so that I was troubled by this.

I went back and looked at the presentation that I missed last meeting that included some chemistry about the dyes, but the upshot of the discussion was that at a future meeting we would have a more in-depth discussion of these kinds of compounds because epidemiology is epidemiology. It has its limitations and I was going back through her presentation and there was a lot about what those limitations were there. I think we have a long way to go before we can use epidemiology to draw real firm conclusions.

DR. ANDERSEN: I think that happy juxtaposition of this going out as a tentative safety assessment, comments, come back in December. I've asked Julie to come back in December to really focus on the

chemistry part that we short-changed at the June meeting and we'll put all of these issues on the table as this one's being readied to go final in December.

DR. BERGFELD: It's timely to do that. We've said we'd do that at least every 2 years or more often especially when the question arises. Thank you very much for bringing that to our attention again.

Report

Draft Final Safety Assessment

2-Amino-4-Hydroxyethylaminoanisole and its Sulfate Salt as Used in Hair Dyes

December 13, 2011

The 2011 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Christina Burnett, Scientific Analyst/Writer.

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INTRODUCTION

This report reviews the available scientific literature, including unpublished data provided by industry, for 2-amino-4-hydroxyethylaminoanisole and its salt, 2-amino-4-hydroxyethylaminoanisole sulfate. These two ingredients are used as coupling agents in oxidative hair dyes.

CHEMISTRY

Definition and Structure

The definitions and structures of these 2 ingredients are presented in Table 1.

2-Amino-4-hydroxyethylaminoanisole and 2-amino-4-hydroxyethylaminoanisole sulfate are commonly used as components of oxidative hair dyes.[1] These ingredients act as "couplers" and react with "precursors." In a typical formulation, a precursor, such as p-phenylenediamine, is activated via an oxidant, such as peroxide. The resultant activated, imino-iminium precursor can then proceed to couple with 2-amino-4-hydroxyethylaminoanisole or 2-amino-4-hydroxyethylaminoanisole sulfate to form a new compound (Figure 1). This in-situ coupled product is purported to be the actual dye that colors the hair in these types of oxidative hair dyes.

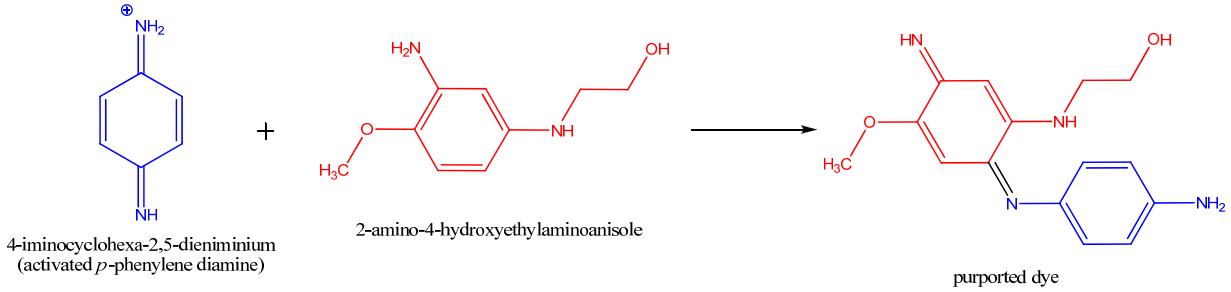


Figure 1. In-situ colorant formation.[1]

N-Nitrosation and Safety Issues

Although nitrosamine content has not been reported, 2-amino-4-hydroxyethylamino-anisole is a secondary amine, and can therefore potentially be nitrosated. Of concern in cosmetics is the conversion (nitrosation) of secondary amines (R1-NH-R2), such as 2-amino-4-hydroxyethylamino-anisole (wherein R1 and R2 are ethanol and methoxyaniline), into *N*-nitrosamines that may be carcinogenic. Of the approximately 209 nitrosamines tested, 85% have been shown to produce cancer in laboratory animals.[2] Nitrosation can occur under physiologic conditions.[3] Depending on the nitrosating agent and the substrate, nitrosation can occur under acidic, neutral, or alkaline conditions. Atmospheric NO_2 may also participate in the nitrosation of amines in aqueous solution.[4] Accordingly, 2-amino-4-hydroxyethylamino-anisole and 2-amino-4-hydroxyethylaminoanisole sulfate should be formulated to avoid the formation of nitrosamines.

Physical and Chemical Properties

The available information on the physical and chemical properties of 2-amino-4-hydroxyethylaminoanisole and its sulfate salt are presented in Table 2.

Method of Manufacture

The manufacture of 2-amino-4-hydroxyethylaminoanisole sulfate can be accomplished via a six step synthesis from commercially available 4-methoxyaniline (Figure 2).[5,6] In the first step, 4-methoxyaniline is acetylated with acetic anhydride. The resultant acetamide is then nitrated with nitric and sulfuric acids. Then, *N*-(4-methoxy-3-nitrophenyl)acetamide is reduced from the amide to the amine, via reflux in hydrochloric acid. Next, the amine is oxidized to a carbamate using beta-chloroethyl chloroformate. Then, the resulting chloroethylcarbamate is heated with potassium hydroxide to produce the alcohol. Finally, the nitro group is reduced to the primary amine over Raney nickel and treated with sulfuric acid to produce the salt, 2-amino-4-hydroxyethylaminoanisole sulfate. The free base, 2-amino-4-hydroxyethylaminoanisole, easily can be prepared by neutralizing this salt.

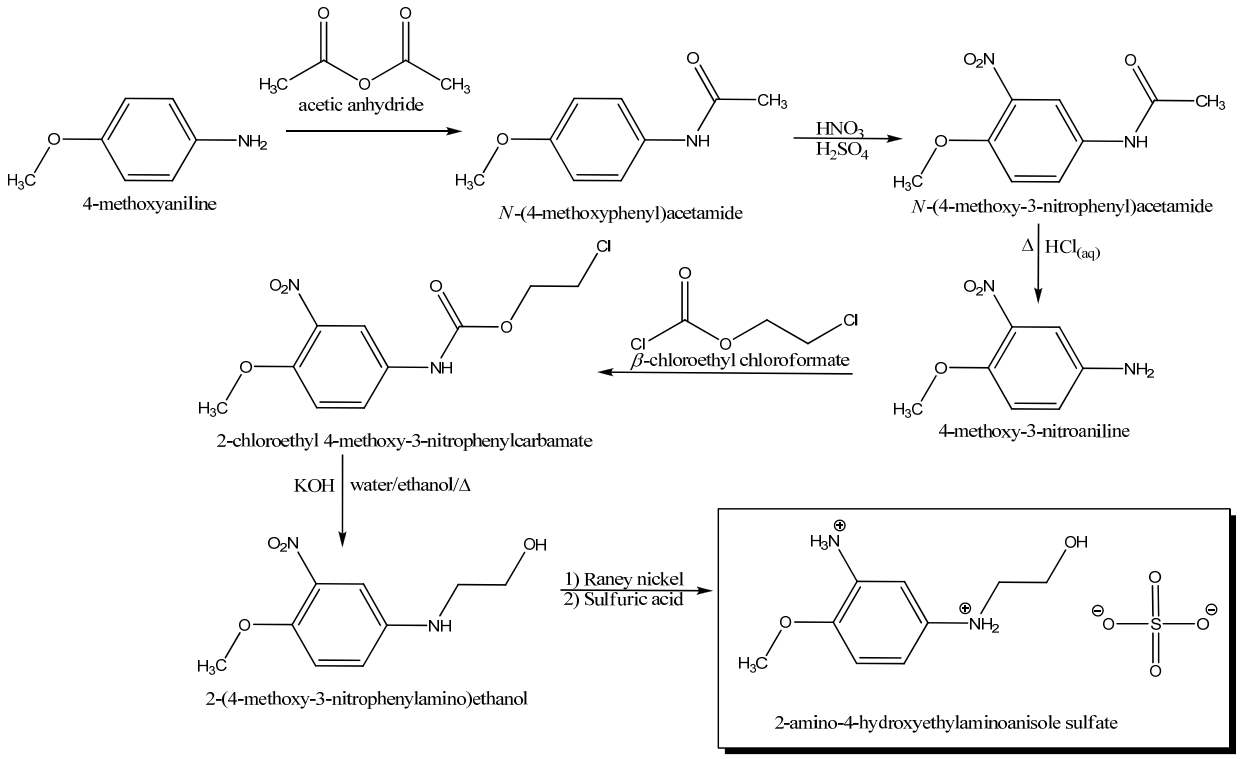


Figure 2. A method of synthesis for 2-amino-4-hydroxyethylaminoanisole sulfate.[5,6]

Impurities

Purity for 2-amino-4-hydroxyethylaminoanisole sulfate has been reported to be 99.3-100% with HPLC at 210-304 nm.[7] Impurities 4-methoxyaniline (a starting material), 4-methoxy-3-nitroaniline (a synthesis intermediate), and 2-methoxy-5-nitroaniline (a by-product) were reported to be below the detection limit of 10 ppm, however, 2,4-diaminoanisole (a by-product) was reported at concentrations of 120-600 ppm in 4 different test batches. Methanol, ethanol, isopropanol, acetone, ethyl acetate, cyclohexane, methyl ethyl ketone, and monochlorobenzene (solvents used for the synthesis) were not detected at 100 ppm (detection limit).

USE

Cosmetic

According to information supplied to the Food and Drug Administration (FDA) by industry as part of the Voluntary Cosmetic Registration Program (VCRP), 2-amino-4-hydroxyethylaminoanisole sulfate is used in a total of 94 hair coloring formulations.[8] In a survey of use concentrations conducted by the Personal Care Products Council, 2-amino-4-hydroxyethylaminoanisole sulfate is used at a concentration range of 0.008-1.5% (maximum 3% before dilution) in hair coloring products.[9] No uses or concentrations were reported for 2-amino-4-hydroxyethylaminoanisole.

According to information provided by the Hair Coloring Technical Committee of the Personal Care Products Council, 2-amino-4-hydroxyethylaminoanisole sulfate is used as an oxidative hair coloring agent.[10] The intended maximum use ('on-head") concentration is 1.5%. This ingredient and a developer would be mixed at ratios between 1:1 to 1:3 (g dye:g hydrogen peroxide) during the hair dying process. It is general practice to apply 100 g of finished mixed hair dye product for approximately 30 minutes before rinsing off with water and shampoo and this process may be repeated on a monthly basis.

The Scientific Committee on Consumer Safety (SCCS) for the European Commission concluded that 2-amino-4-hydroxyethylaminoanisole sulfate would not pose a health risk to the consumer when used as an ingredient in oxidative hair dye formulations at a maximum concentration of 1.5%.[7] The SCCS could not exclude the possibility that this ingredient may be sensitizing. The Committee also determined that because 2-amino-4-hyroxyethylaminoanisole sulfate is a secondary amine, it should not be used with nitrosating substances and the nitrosamine content in the ingredient should be < 50 ppb.

Hair Dye Caution Statement - FDA labeling

These ingredients are considered coal tar hair dyes 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 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 a caution statement and patch test instructions for determining whether the product causes skin irritation. The CIR Expert Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 hours after application of the test material and prior to the use of a hair dye formulation.

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

Dermal/Percutaneous

In an in vitro percutaneous absorption study, [^{14}C] 2-amino-4-hydroxyethylaminoanisole sulfate at 1.5% as part of an oxidative hair dye formulation in the presence of hydrogen peroxide and a reaction partner was applied to excised, dermatomed pig skin.[11] The integrity of the skin was tested by measuring trans-epidermal water loss (TEWL) prior to test material application. The test substance (100 μg/cm^2) was applied to the skin samples for 30 minutes and then washed off with water and shampoo. Measurements for radioactivity in the receptor fluid were made at 16, 24, 40, 48, 64, and 72 h after application. At experiment end, the skin was heat-treated and the upper skin (stratum corneum + upper stratum germinativum) was mechanically separated from the lower skin (lower stratum germinativum + upper epidermis). Most of the test material was found in the rinsing solution (mean value $\pm$ SD = 1340.92 $\pm$ 50.57 μg/cm^2; 92.69%). The test material was also found in upper skin (2.352 $\pm$ 0.824 μg/cm^2; 0.162%), the lower skin (0.303 $\pm$ 0.219 μg/cm^2; 0.021%), and the fractions of receptor fluid collected over 72 h (0.409 $\pm$ 0.223 μg/cm^2; 0.028%). Total recovery of the radiolabeled 2-amino-4-hydroxyethylaminoanisole sulfate was 1446.71 $\pm$ 7.86 μg/cm^2 (96.59%). The total amount of radiolabeled 2-amino-4-hydroxyethylaminoanisole sulfate that was biologically available was 3.064 $\pm$ 0.88 μg/cm^2 (0.211%). The SCCS found that this study did not follow its requirements and performed a worst case scenario calculation that incorporated 2 standard deviations for dermal absorption of 2-amino-4-hydroxyethylaminoanisole sulfate, which yielded a value of 4.82 μg/cm^2.[7]

In an in vivo percutaneous absorption study, [^{14}C] 2-amino-4-hydroxyethylamino-anisole dihydrochloride was applied to the skin of Sprague-Dawley rats.[12] Groups of 3 male and 3 female rats received the test material at 0.75% in a commercial hair dye with hydrogen peroxide, 0.75% in a commercial hair dye without hydrogen peroxide, or in a 3.47% aqueous solution on a 9 cm^2 area. The mean dose for all 3 applications was 0.83 mg/cm^2 free base. Application time was 30 minutes, after which the formulations were scraped off and the test sites were rinsed with approximately 100 ml 3% shampoo solution and water. The areas were then covered to prevent the animals from licking the test sites during a 72 h observation period. Urine and feces were collected daily and the rats were killed 72 h after application. The application sites, blood, and several organs were collected and analyzed for radioactivity. After complete removal of the skin, the radioactivity in the carcass was measured.

The mean recovery rates for the free base at 0.75% in a commercial hair dye with hydrogen peroxide, 0.75% in a commercial hair dye without hydrogen peroxide, and in a 3.47% aqueous solution were 97.7%, 95.1%, and 99.9%, respectively. Most of the applied dose (93.6-98.9%) was recovered in the rinse solutions. At the cutaneous application site, 1.51%, 0.57%, and 0.75% of the applied formulation with hydrogen peroxide, without hydrogen peroxide, and in aqueous solution were recovered, respectively. Absorbed material was mainly excreted via the urine at 0.02%, 0.097%, and 0.180% of the applied formulation with hydrogen peroxide, without hydrogen peroxide, and in aqueous solution, respectively, and a very small amount was excreted in the feces: 0.008%, 0.027%, and 0.047%, respectively. The amount of test material in the carcasses and organs was close to or below the limit of detection for all 3 dose groups (0.005-0.008%). From these observations, it was determined that the absorption rates were 1.54% (12.8 μg/cm^2), 0.70% (5.8 μg/cm^2), and 0.99% (8.2 μg/cm^2) for the formulations with hydrogen peroxide, without hydrogen peroxide, and in aqueous solution. Using the highest absorption rate (12.8 μg/cm^2 as free base), the equivalent penetration rate for 2-amino-4-hydroxyethylaminoanisole sulfate is 19.7 μg/cm^2. This study was reported to not be GLP compliant.[7]

To the limit of detection, no parent compound or metabolites were found in the blood of 5 female volunteers after application by a professional hair dresser for 15 min. of 2-amino-4-hydroxyethylamino-anisole sulfate plus hydrogen peroxide oxidizing agent, containing 2.2% of the dye, indicating that less than 1.6 μg/cm² became bioavailable.[7]

Other Dose Administration

In an in vitro study, human intestinal epithelial (TC-7) cells were used to determine the bioavailability of 2-amino-4-hydroxyaminoanisole sulfate across the intestinal barrier.[14] Analysis of the donor (apical) and receiver (basolateral) samples was done using HLPC-MS/MS and the apparent permeability coefficient (P_{app}) was calculated for 2 independent experiments. ^{14}C-mannitol (~4 μM) was used to demonstrate the integrity of the cell monolayer. Only monolayers revealing a permeability of < 2.5 x 10^{-6} cm/sec were used. According to the laboratory's classification system, a P_{app} < 2x10^{-6} cm/sec indicates low permeability. Ranitidine, which has a 50% absorption in humans, was used as a low permeability reference compound, and it and another reference compound, propranolol, were well within the acceptance range and validated the

study. The P_{app} for 2-amino-4-hydroxyethylaminoanisole sulfate was 73.3 x 10^{-6} cm/sec, which equates to a high permeability classification ($P_{app} \geq 20x\ 10^{-6}$ cm/sec) by this laboratory's classification system. It was concluded that 2-amino-4-hydroxyethylaminoanisole is readily absorbed in the gastrointestinal tract after oral administration.[7]

TOXICOLOGICAL STUDIES

Acute Toxicity

Oral – Non-Human

In an acute oral toxicity study, doses of 2-amino-4-hydroxyethylaminoanisole sulfate were administered to Wistar rats and CF 1 mice.[15] The dose groups for the rats (5 rats per sex per dose group) and mice (10 females per dose group) were 250, 375, 500, 625, and 750 mg/kg body weight. There was an additional dose group of 875 mg/kg body weight in the mice. Doses were established after a range finding study in mice found the median lethal dose to be less than 875 mg/kg body weight. The test material was administered once by oral gavage. Mortality and clinical signs of toxicity were recorded during the 14 day observation period. Body weights were recorded weekly. All animals were necropsied. Clinical signs of toxicity observed after dosing included tonic spasm, piloerection, and higher respiratory rate in both rats and mice. Three male and 2 female rats and 2 mice in the 375 mg/kg, 4 male and 2 female rats and 6 mice in the 500 mg/kg, all male and 4 female rats and 5 mice in the 625 mg/kg, and all male and female rats and 9 mice in the 750 mg/kg dose groups died between the first 24 h and 6 days after dosing. Additionally, all mice in the 875 mg/kg dose group died. No macroscopic changes were noted at necropsy. No data were provided regarding control groups. The LD_{50} of 2-amino-4-hydroxyethylaminoanisole sulfate in male rats, female rats, and female mice were calculated to be 475, 588, and 538 mg/kg body weight, respectively. This study was reported to not be GLP compliant.[7]

In another acute oral study, groups of 5 male and 5 female NMRI white mice received 125, 250, 500, 750, or 1000 mg/kg 2-amino-4-hydroxyethylaminoanisole sulfate by gavage.[16] Doses were established after a range finding study in mice found the median lethal dose to be less than 2000 mg/kg body weight. The test material was administered as a 1.25-10% dilution in deionized water. Mortality and clinical signs of toxicity were recorded during the 14 day observation period. Clinical signs of toxicity included those related to the central nervous system, coordination, reflexes, and autonomic functions with dose-dependent severity up to 72 h after administration. Weight gains were reduced in all surviving animals. One male and 1 female in the 125 mg/kg, 2 males and 1 female in the 250 mg/kg, 4 males and 3 females in the 500 mg/kg, 3 males and all females in the 750 mg/kg, and all males and 4 females in the 1000 mg/kg dose groups died within 24 to 72 h of dosing. No macroscopic changes were noted at necropsy. No data were provided regarding control groups. The calculated LD_{50} of 2-amino-4-hydroxyethylaminoanisole sulfate in male mice was 333 mg/kg body weight and in female mice was 351 mg/kg body weight. An LD_{50} for male and female mice combined was calculated to be 327 mg/kg body weight.

Repeated Dose Toxicity

Dermal – Non-Human

The dermal toxicity potential of 2-amino-4-hydroxyethylaminoanisole sulfate was evaluated in a 28 day study in SPF Pirbright White guinea pigs.[17] Dose groups of 5 animals of each sex and received 0, 50, 150, or 300 mg/kg body weight of the test material in tap water at a dose volume of 1 ml/kg body weight. The test material was applied once daily to a 3 x 4 cm area on clipped dorsal skin. The animals were checked twice daily for mortality. Clinical signs of toxicity were recorded daily and body weights were recorded weekly. Complete hematology and blood chemistry investigations and urinalysis were performed on day 0 and day 28. All animals were killed at the end of the treatment period. Select organs were weighed and a detailed necropsy was performed in all animals. Hearts and kidneys of the control and high dose group animals were studied histopathologically. Additionally, all gross lesions observed and the liver and skin of all dose groups were examined microscopically.

No deaths occurred and no relevant clinical signs were observed, including any signs of erythema or edema. Body weight gains were comparable to the control group. No treatment-related changes were observed in hematology, blood chemistry parameters, or urinalysis. No gross lesions were noted at necropsy and organ weights were comparable to the control group. The NOAEL for this 28-day dermal study of 2-amino-4-hydroxyethylaminoanisole sulfate was 300 mg/kg body weight.[17]

Oral – Non-Human

The potential for oral toxicity to 2-amino-4-hydroxyethylaminoanisole sulfate was investigated in Wistar HanBrl:WIST (SPF) rats.[18] Dose groups were comprised of 15 animals of each sex and received 0, 15, 50, or 200 mg/kg body weight of the test material in distilled water at a dose volume of 10 ml/kg body weight. The test material was administered by oral gavage once daily for 108/109 days. Clinical signs of toxicity, food consumption, and body weights were recorded weekly. Ophthalmoscopic exams were performed before and after the treatment period. At week 15, functional observational battery, locomotor activity, and grip strength were tested, as were thyroid hormone levels (5 rats/sex/dose). Complete hematology and blood chemistry investigations and urinalysis were performed at the completion of the dosing period. All animals were killed at the end of the treatment period. Select organs were weighed and a detailed necropsy was performed in all animals. Histological exams were performed on organs and tissues of the control and high dose group animals, and on all gross lesions from all animals. Additionally, thyroids, spleens, kidneys, and pituitary glands were examined in the mid and low dose groups.

All animals survived until necropsy. Blue discoloration of the urine was noted in the female rats of the 15 mg/kg dose group as well as all animals in the 50 and 200 mg/kg dose groups. No clinical signs of toxicity were observed. No irregularities were observed during the functional observational battery or ophthalmoscopic exams. There were no treatment-related changes in food consumption or body weight gains. Thyroid hormone levels were comparable to the control group.

In the 200 mg/kg body weight dose group, both sexes had slight anemia with compensatory reticulocytosis that presented as lower red blood cell counts, lower hemoglobin, elevated methemoglobin, lower hematocrit levels, and elevated reticulocyte counts and maturity indices. Reduced creatinine, elevated triglyceride sodium and chloride concentrations, slight proteinuria and increased bilirubin and nitrite was observed in both sexes of this dose group as well. Marginally elevated thyroid-to-brain weight ratios were observed in both sexes and, in females, elevated mean absolute and relative liver, kidney, and spleen weights were noted. Follicular cell enlargement of the thyroid gland was observed in both sexes of the 200 mg/kg dose group. Additionally, pigment storage and tubular swelling with necrosis of tubular cells and basal membrane thickening was observed in the kidneys, and increased mean grade of extra medullary hemopoiesis was observed in the spleen. Slight hypertrophy of chromophobic cells of the pituitary gland was seen in males of the 200 mg/kg dose group. Similar adverse effects were observed in the 50 mg/kg body weight dose group, but occurred mostly in the female rats. No adverse effects were noted in the hematology, blood chemistry, or gross necropsy of the 15 mg/kg body weight dose group. Due to the slight anemia and morphological and histological changes of the thyroid gland, kidneys, and pituitary gland in the 50 mg/kg dose group, the NOAEL was determined to be 15 mg/kg body weight of 2-amino-4-hydroxyethylaminoanisole sulfate in this 15 week rat study.[18]

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In a teratogenicity study, mated female Wistar HanBrl: WIST (SPF) rats received 2-amino-4-hydroxyethylaminoanisole sulfate by gavage on days 6-20 of gestation.[19] The doses used were based on the results of a range finding study. In the main study, groups of 22 rats received 0, 10, 30, or 150 mg/kg body weight of the test material in bi-distilled water. Maternal clinical signs were monitored twice daily. Body weights were recorded daily and food consumption was measured over 3-day periods. Dams were killed on gestation day 21. Complete necropsy and macroscopic examination of the organs was performed. Gravid uterus weights were determined and fetuses were removed, sexed, weighed, and examined externally. Implantation sites, resorption sites, and live and dead fetuses were recorded. Half of the fetuses were examined for soft-tissue abnormalities and half for skeletal abnormalities.

One animal in the high dose group was found dead on gestation day 10. The death was considered the result of a dosing error. No other treatment-related clinical signs of toxicity were observed. Urine of the 30 and 150 mg/kg dose groups was darkly discolored. A slight decrease in mean food consumption was observed for the entire treatment period in the 150 mg/kg dose group. This group also had slightly reduced body weight gain up to gestation day 16. No maternal treatment-related effects were observed at gross necropsy. There were also no treatment-related effects observed viscerally or skeletally in the fetuses. Uterus and placenta weights, number of corpora lutea, and implantations were similar to controls in all dose groups. Litter size, fetal mortality, fetal body weight, and sex ratio were also comparable to the controls. The maternal NOAEL was 30 mg/kg body weight and the fetal NOAEL was 150 mg/kg body weight in this rat teratology study.[19]

GENOTOXICITY

In Vitro

The mutagenic potential of 2-amino-4-hydroxyethylaminoanisole sulfate was studied in an Ames test using *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537, with and without S9 metabolic activation.[20] The test concentrations were 33, 100, 333, 1000, 2500, or 5000 µg/plate. The positive controls were 4-nitro-o-phenylenediamine, sodium azide, methyl methane sulfonate, and 2-aminoanthracene. Reduced background growth was observed at 2500 and 5000 µg/plate. No biologically relevant increases in revertant colony numbers were observed in any test strain at any dose level, with or without metabolic activation. Controls yielded expected results. It was concluded that 2-amino-4-hydroxyethylaminoanisole sulfate was not mutagenic in this assay.

The mutagenic activity of 2-amino-4-hydroxyaminoanisole sulfate was studied in mouse lymphoma L5178Y $TK^{+/-}$ cells at the *tk* locus.[21] After a range-finding test to measure cytotoxicity, an independent experiment was performed. The concentrations for the main experiment ranged from 0.5-100 µg/ml without S9 metabolic activation (precipitation was observed without S9 at $\geq$ 50 µg/ml) and 1.0-500 µg/ml (with S9 metabolic activation). The vehicle control was culture medium. The positive controls were benzo[α]pyrene with S9 and ethylmethanesulfonate without S9. The cultures were incubated with the test material for 4 h. Mutant frequency and cell survival were determined as was as the size/optical density of colonies and the ratio of small versus large colonies. A relative total growth of 20.61% compared to the control was observed in the 500 µg/ml with S9, while without S9, the relative total growth was 23.09% in 100 µg/ml. A biologically significant increase in the number of mutant colonies was observed with and without S9. With S9, the highest mutation factor was 2.32 at 500 µg/ml and the historical control range was exceeded at concentrations of 50 µg/ml and greater. Without S9, the highest mutation factor was 2.13 at 100 µg/ml. A biologically relevant shift towards small colonies indicating a clastogenic effect was observed following treatment with the test material, with and without metabolic activation. The

controls yielded expected results. It was concluded that 2-amino-4-hydroxyethylaminoanisole sulfate was mutagenic in this mouse lymphoma assay.

The genotoxic potential of 2-amino-4-hydroxyethylaminoanisole sulfate was studied in a micronucleus study using human peripheral blood lymphocytes.[22] The test material was tested at concentrations of 25, 100, and 150 µg/ml with S9 metabolic activation and at concentrations of 3, 5, and 8 µg/ml without S9 metabolic activation. The positive controls were cyclophosphamide with S9 and 4-nitroquinoline-1-oxide and vinblastine without S9. Cells were incubated with the test material 24 h after mitogen stimulation with phytohemagglutinin. Incubation for cells with metabolic activation was 3 h and 20 h for cells without metabolic activation. Cells were harvested 72 h after mitogen stimulation. The replication index (RI) was calculated from the proportions of mononucleate, binucleate, and multinucleate cells in 500 cells per replicate. One thousand binucleate cells from each culture were analyzed for the occurrence of micronuclei.

The RI at the highest concentration tested with and without metabolic activation were 63% and 68%, respectively. With S9, a concentration-related increase in the frequency of micronucleated binucleate (MNBN) cells was observed with statistical significance at 100 and 150 µg/ml. The frequency of MNBN also exceeded historical control range in single cultures in the same concentrations. Without S9, the frequencies of MNBN were similar to concurrent controls at all concentrations and within the historical range for vehicle controls. A small, borderline increase in the frequency of MNBN at 8 µg/ml was observed that exceeded historical vehicle controls in one culture only. The effect was considered equivocal due to the high level of cytotoxicity (68%) at this concentration. In this study, 2-amino-4-hydroxyethylaminoanisole sulfate was considered to be genotoxic.[22]

In Vivo

The genotoxic potential of 2-amino-4-hydroxyethylaminoanisole sulfate was studied in a micronucleus test using NMRI mice.[23] A dose range finding experiment preceded the main study. In the main study, groups of 5 mice of each sex received single intraperitoneal injections of 0, 20, 100, or 200 mg/kg body weight 2-amino-4-hydroxyethylaminoanisole sulfate in distilled water, with an additional group of mice receiving the 200 mg/kg dose. Control groups received distilled water or 40 mg/kg body weight cyclophophamide in 0.9% NaCl. Bone marrow cells were collected at 24 or 48 h (for the 200 mg/kg dose group). At least 2000 polychromatic erythrocytes per animal were analyzed, and the ratio between polychromatic and total erythrocytes per animal was determined by counting at least 200 immature polychromatic erythrocytes per animal. In the dose range finding study, toxic effects observed at 200 mg/kg included palpebral closure and lethargy within the first hour of treatment. Also in the range finding study, a dose of 400 mg/kg caused mortality, and signs of systemic toxicity were observed at both 400 mg/kg and 200 mg/kg. In the main study, no treatment-related mortalities or clinical signs of toxicity were observed. There was no statistically significant increase in micronuclei in the treatment groups when compared to the controls. The study concluded that up to 200 mg/kg body weight 2-amino-4-hydroxyethylaminoanisole sulfate was not genotoxic in this micronucleus assay.

The potential for 2-amino-4-hydroxyethylaminoanisole sulfate to induce unscheduled DNA synthesis (UDS) was assessed using male Wistar rats.[24] A dose range finding experiment preceded the main study. In the main study, groups of 5 rats received single oral doses of 75 or 750 mg/kg body weight 2-amino-4-hydroxyethylaminoanisole sulfate in bi-distilled water. Control groups received 10 ml/kg body weight bidistilled water or 100 mg/kg body weight 2-acetylaminofluorene. Rats were killed either 4 h after treatment (one group of the 750 mg/kg dose group) or 16 h after treatment (the 75 mg/kg dose group and the additional 750 mg/kg dose group). Liver perfusion was performed. At least 5 primary hepatocyte cultures were made from each animal and exposed for 4 h to 3H-thymidine. The dye-exclusion method was utilized to determine if any liver cell toxicity occurred. After the radiolabel exposure, the cells were washed and slides were prepared. At least 2 slides per animal were evaluated from the occurrence of UDS for 3 animals per dose group, which equated to 100 cells/animal. Heavily labeled S-phase cells were excluded from counting, and background grains were subtracted from the grains observed from the nucleus to obtain relevant net nuclear grains.

In the range finding study, mortality occurred at 1000 mg/kg within 24 h of dosing. Survivors in this group had reduced spontaneous activity, eyelid closure, and piloerection. At 750 mg/kg, there was no mortality. The kidneys, urine, and liver of the rats showed dark discoloration. In the main study, no mortality or clinical signs of toxicity were observed. No UDS induction was observed in the hepatocytes of the treated animals at any dose level or time period when compared to controls. There was no increase in the number of nuclear grains or the resulting net grains at either dose or time period. The study concluded that 2-amino-4-hydroxyethylaminoanisole sulfate did not induce DNA damage in this UDS assay.[24]

IRRITATION AND SENSITIZATION

Irritation

Dermal – Non-Human

In a dermal irritation study, 0.5 g of a commercial hair dye formulation that contained 3% 2-amino-4-hydroxyethylaminoanisole sulfate was applied to shaved skin (~ 6 cm^2) of 3 New Zealand White rabbits.[25] The test sites were then semi-occluded for 4 h, after which the test material was washed off with water. The skin was evaluated for reactions at 30 min, 1, 24, 48 and 72 h and then daily up to 14 days after the material was removed. Slight erythema (scores of 1 and 2) and very slight edema (score of 1) were recorded at several observation periods. These effects had completely disappeared within 7 days. In each rabbit, the mean 24/48/72 h scores for erythema and edema were 1.33 and 1.0; 1.67 and

1.0; and 1.33 and 1.0, respectively. The study concluded that 2-amino-4-hydroxyethylaminoanisole sulfate was a mild transient skin irritant when tested at 3%.

In another dermal irritation study, 1% 2-amino-4-hydroxyethylaminoanisole sulfate suspended in gum Arabic was applied to 10 female SPF white guinea pigs.[26] The test material was applied with a brush 3 times a day for 20 min durations on 2 consecutive days on the left and right clipped flanks of the animals. The treatment period was followed by a 3-day observation period. Any effects were scored according to the Draize method. Very slight erythema was observed in 2 of the animals on the second treatment day. No edema was observed. It was concluded that 2-amino-4-hydroxyethylaminoanisole sulfate at 1% was essentially non-irritating. This study was reported to not be GLP compliant.[7]

Ocular

A hair dye formulation containing 3% 2-amino-4-hydroxyethylaminoanisole sulfate was tested for ocular irritation potential in 3 New Zealand White rabbits.[27] The conjunctival sac of one eye of each rabbit was instilled with 0.1 ml of the test substance and not rinsed. The untreated eye served as a control. Both eyes were examined at 1, 24, 48, and 72 h post-treatment according to the Draize method. No cornea or iris effects were noted at any observation time. Slight conjunctival redness (score of 1) was noted at 24 h in all animals, but these effects were gone within 3 days of treatment. In each rabbit, the mean 24/48/72 h scores for conjunctival erythema were 0.67, 0.33, and 0.67, respectively. The study concluded that 3% 2-amino-4-hydroxyethylaminoanisole sulfate was a transient and mild irritant in the eyes of rabbits.

In another ocular irritation study, a 1% aqueous solution of 2-amino-4-hydroxyethylaminoanisole sulfate was tested in 10 female Pirbright White (SPF) guinea pigs.[28] The conjunctival sac of the right eye of the guinea pigs was instilled with 0.1 ml of the test substance and not rinsed. The left eye of the animals was left untreated and served as a control. At 24 h after application, the eyes were washed with fluorescein-sodium solution. Redness and discharge was observed in 5 of the guinea pigs 30 minutes after treatment. Redness was also observed in 2 animals at 7 h post-treatment, but this effect cleared at 24 h. No other ocular effects were observed. It was concluded that 1% 2-amino-4-hydroxyethylaminoanisole sulfate caused transient conjunctival irritation in this study. It should be noted that this study was reported to not be GLP compliant.[7]

Sensitization

Dermal – Non-Human

A local lymph node assay (LLNA) was performed using 2-amino-4-hydroxyethylaminoanisole sulfate dissolved in DMSO.[29] CBA/Ca female mice were divided into groups of 5 and received 0.25%, 0.5%, 1% or 2% of the test material on the ear surface (25 µl) once daily for 3 consecutive days. After each application, the ears were dried with a hair dryer for ~5 min. A positive control group received 0.25%, 0.5%, 1%, or 2% p-phenylenediamine in DMSO. Five days after the initial topical treatment, all animals were injected intravenously with 250 µl phosphate buffered saline containing 20 µCi of [^{3}H] methyl thymidine. Approximately 5 h after injection, the animals were killed and the auricular lymph nodes were excised. Single-cell suspensions were prepared from pooled lymph nodes, with the cells precipitated by trichloroacetic acid (TCA), and radioactivity measured by liquid scintillation. The stimulation indices (SI) were calculated.

No clinical signs of toxicity or deaths occurred during the treatment period in any dose group. The SI were1.29, 1.03, 1.12, and 1.42 for the 0.25%, 0.5%, 1%, and 2% dose groups, respectively. The estimated concentrations for a SI of 3 (EC_3) could not be calculated. The positive control group produced expected results and validated the study. The study concluded that 2-amino-4-hydroxyethylaminoanisole sulfate tested up to 2% in DMSO was not a skin sensitizer.[29]

QSAR

ATOPS-MODE quantitative structure-activity relationship (QSAR) model was utilized to predict the sensitization potential of all hair dye ingredients registered in Europe (229 substances as of 2004). [30] The model predicted 2-amino-4-hydroxyethylaminoanisole to be a weak sensitizer. The sensitization potential of the sulfate salt was not evaluated.

CLINICAL USE

Epidemiology

2-Amino-4-hydroxyethylaminoanisole and its sulfate salt are oxidative hair dye ingredients. While the safety of individual hair dye ingredients are not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. A detailed summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/findings.shtml.

RISK ASSESSMENT

The SCCS calculated the margin of safety for 2-amino-4-hydroxyethylaminoanisole sulfate to be 267.[7] To obtain this value, the systemic exposure dose (SED) of 0.056 mg/kg was determined based on the maximum absorption through the skin (4.82 µg/cm^2= the mean $\pm$ 2SD from an in vitro percutaneous absorption study), typical body weight (60 kg) and skin surface area (700 cm^2 for scalp surface area), and dermal absorption per treatment (3.374 mg, calculated using the values for maximum absorption and average body weight and skin surface area assumptions), which was then divided into the NOAEL of the 15 week rat (repeated dose oral toxicity) study (15 mg/kg).

SUMMARY

The cosmetic ingredients 2-amino-4-hydroxyethylaminoanisole and its salt, 2-amino-4-hydroxyethylaminoanisole sulfate, are used as coupling agents in oxidative hair dyes. The free base currently has no reported uses by the FDA or the cosmetics industry. The sulfate salt is used in a total of 94 hair coloring formulations at a concentration range of 0.008-1.5%.

In an in vitro percutaneous absorption study in dermatomed pig skin, 3.064 $\pm$ 0.88 μg/cm^2 (the mean $\pm$ SD; 0.211%) of 1.5% radiolabeled 2-amino-4-hydroxyethylaminoanisole sulfate in an oxidative hair dye formulation with a reaction partner was found to be biologically available. An in vivo study in rats found the equivalent penetration rate of radiolabeled 2-amino-4-hydroxyethylaminoanisole sulfate, which was calculated from the measured penetration rate of the free base, to be 19.7 μg/cm^2. A bioavailability study of 2-amino-4-hydroxyethylaminoanisole sulfate in human intestinal epithelial cells concluded that this chemical was readily absorbed in the gastrointestinal tract after oral administration.

An acute oral toxicity study of 2-amino-4-hydroxyethylaminoanisole sulfate determined LD_{50} values of 475 mg/kg and 588 mg/kg for male and female rats, respectively. The same study calculated a LD_{50} of 538 mg/kg in female mice. Clinical signs of toxicity observed after dosing included tonic spasm, piloerection, and higher respiratory rate in both rats and mice. In another acute oral toxicity study, the calculated total LD_{50} of 2-amino-4-hydroxyethylaminoanisole sulfate in male and female mice combined was 327 mg/kg.

The NOAEL for a 28-day dermal study of 2-amino-4-hydroxyethylaminoanisole sulfate in guinea pigs was 300 mg/kg body weight, which was the highest dose tested. In a 15-week oral study in rats, the NOAEL was determined to be 15 mg/kg body weight 2-amino-4-hydroxyethylaminoanisole sulfate. Slight anemia and morphological and histological changes to the thyroid gland, kidneys, and pituitary gland were observed at higher dose levels.

In a teratogenicity study where female rats received 2-amino-4-hydroxyethylaminoanisole sulfate by gavage at doses of 0, 10, 30, or 150 mg/kg body weight of the test material in bi-distilled water, the maternal NOAEL was 30 mg/kg body weight and the fetal NOAEL was 150 mg/kg body weight. Dams in the high dose group experienced effects on food consumption and body weight gains.

The ingredient 2-amino-4-hydroxyethylaminoanisole sulfate was not mutagenic in an Ames assay, but was found to be mutagenic in an in vitro mouse lymphoma assay and in a micronucleus study using human peripheral blood lymphocytes. In in vivo studies, 2-amino-4-hydroxyethylaminoanisole sulfate was not genotoxic in a mouse micronucleus test and it did not induce DNA damage in a UDS assay in rats.

In dermal irritation studies, 2-amino-4-hydroxyethylaminoanisole sulfate at 1% was essentially non-irritating to guinea pig skin; however, mild transient skin irritation was observed at 3% in rabbits. At these concentrations, 2-amino-4-hydroxyethylaminoanisole sulfate was a transient and mild irritant in the eyes of rabbits and guinea pigs. A local lymph node assay (LLNA) study concluded that 2-amino-4-hydroxyethylaminoanisole sulfate tested up to 2% in DMSO was not a skin sensitizer.

A QSAR model predicted that 2-amino-4-hydroxyethylaminoanisole to be a weak sensitizer.

The most recent CIR review of available epidemiology studies concluded that there is insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers.

A margin of safety calculation for 2-amino-4-hydroxyethylaminoanisole sulfate by the EU's SCCS yielded a value of 267 compared to the NOAEL for repeated dose oral toxicity in rats.

DISCUSSION

The Expert Panel noted gaps in the available safety data for the free base, 2-amino-4-hydroxyethylaminoanisole, in this safety assessment. The available data on the sulfate salt are sufficient, however, and similarity between structural activity relationships and cosmetic function in cosmetic concentrations of use can be extrapolated to support the safety of the entire group.

The Expert Panel recognizes that 2-amino-4-hydroxyethylaminoanisole and its sulfate salt are used as hair dye ingredients and that limited irritation and sensitization data are available. However, hair dyes containing these ingredients, as coal tar hair dye products, are exempt from certain adulteration and color additive provisions of the Federal Food, Drug, and Cosmetic Act, when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Expert Panel expects that following this procedure will identify prospective individuals who would have an irritation/sensitization reaction and allow them to avoid significant exposures.

In considering hair dye epidemiology data, the CIR Expert Panel concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer or other toxicologic endpoints, based on lack of strength of the associations and inconsistency of findings.

CONCLUSION

The CIR Expert Panel concluded that 2-amino-4-hydroxyethylaminoanisole and 2-amino-4-hydroxyethylaminoanisole sulfate are safe for use in hair dye formulations. Were the free base (2-amino-4-hydroxyethylaminoanisole) to be used in the future, the expectation is that it would be used at concentrations similar to the sulfate salt. The Expert Panel cautions that these ingredients should not be used in cosmetic products in which *N*-nitroso compounds are formed.

TABLES AND FIGURES

Table 1. Names, CAS Registry Numbers, Definitions, and Structures of the Diaminoanisole Ingredients.

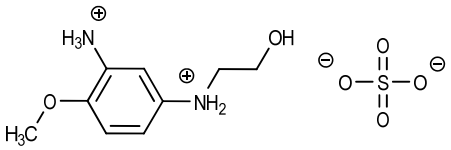
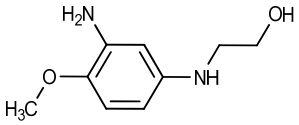
Ingredient CAS No.	Definition	Formula/structure
2-Amino-4-Hydroxyethylaminoanisole Sulfate 83763-48-8	2-Amino-4-Hydroxyethylaminoanisole Sulfate is sulfate salt of a 2,4-diamino-substituted aromatic, anisole, wherein the amine at the 4-position is substituted with ethanol.	
2-Amino-4-Hydroxyethylaminoanisole 83763-47-7	2-Amino-4-Hydroxyethylaminoanisole a 2,4-diamino-substituted aromatic, anisole, wherein the amine at the 4-position is substituted with ethanol.	

Table 2. Chemical properties

Property	Value	Reference
2-amino-4-hydroxyethylaminoanisole		
Molecular Weight g/mol	182.22	[31]
Molecular Volume cm^3/mol @ 20 °C and 760 mmHg	149.1	[31]
Density g/ cm^3 @ 20 °C and 760 mmHg	1.221	[31]
Vapor pressure mmHg@ 25 °C	3.54E-7	[31]
Boiling Point °C	401.8	[31]
logP @ 25 °C	-0.916	[31]
Disassociation constant pKa @ 25 °C	14.68	[31]
2-amino-4-hydroxyethylaminoanisole sulfate		
Physical Form	Powder	[7]
Color	Grey-blue	[7]
Molecular Weight g/mol	280.3	[7]
Density g/ cm^3 @ 20 °C	1.541	[7]
Vapor pressure mmHg @ 20 °C	1.5E-9	[7]
Melting Point °C	138-149	[7,31]
Water Solubility g/L @ 20 °C & pH 2.3	81.99	[7]
Acetone: Water Solubility g/L @ pH 2.1	>5	[7]
DMSO Solubility g/L	>90	[7]
Ethanol Solubility g/L	<10	[7]
logP @ 25 °C pH 7.51	0.59	[7]

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Data

Personal Care Products Council
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Memorandum

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

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

DATE: September 23, 2011

SUBJECT: Comments on the Draft Report on 2-Amino-4-Hydroxyanisole and its Sulfate Prepared for the September 26-27, 2011 CIR Expert Panel Meeting

This report only has numbers on the odd pages.

p.3 - The discussion of the in vitro dermal penetration study should clearly state that it was completed in the presence of a reaction partner.

p.7 - The following sentence in the Summary does not make sense. "In an in vitro percutaneous absorption study in dermatomed pig skin, 3.064 ± 0.88 μg/cm^2 (the mean ± SD; 0.211%) of 1.5% radiolabeled 2-amino-4amino-4-hydroxyethylaminoanisole sulfate was found to be biologically available." From this sentence it is not clear that 1.5% represents the concentration of2-Amino-4-Hydroxyethylaminoanisole Sulfate in an oxidative hair dye formulation that included a reaction partner.

p.7 - In the description of the in vivo dermal penetration study, it is not clear what is meant by "equivalent". In the Summary, it should be made clear that the penetration rate of the sulfate was calculated from the measured penetration rate of the free base.

p.9, Table 1 - Please include references for this table. As this is a review of cosmetic ingredients, the definitions in the International Cosmetic Ingredient Dictionary and Handbook should also be given in Table 1. For ingredients defined by their structure, it would be appropriate to add a footnote to indicate which ingredients are defined only by their structure in the Dictionary.

p.9, Table 2 - Inclusion of trade names in this table is not necessary.

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Personal Care Products Council
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Memorandum

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

FROM: Hair Coloring Technical Committee of the Personal Care Products Council

DATE: November 1, 2011

SUBJECT: Comments on the Tentative Safety Assessment on 2-Amino-4-Hydroxyethylaminoanisole and its Sulfate Salt as Used in Cosmetics

Please consider changing the title to "As Used in Hair Dyes". The current title ("As Used in Cosmetics") suggests that these ingredients may be used in products other than hair dyes.

p.3, last paragraph in the section titled Dermal/Percutaneous - It is inappropriate for the safety assessment of 2-Amino-4-Hydroxyethylaminoanisole and its Sulfate to include a discussion of exposure to reaction products of oxidative hair dyes in the section on Toxicokinetics. If the CIR Expert Panel wants to address the topic of reaction products of oxidative hair dyes, a "boiler plate" statement should be developed. This statement should justify why the approach to ingredient safety assessments for oxidative hair dyes should focus on the ingredients themselves rather than the reaction products. Exposure to reaction products is considerably lower. Note, that this is the conclusion in the SCCS opinion - see the first bullet point in the conclusion of the 2010 SCCS opinion cited in the CIR report.

p.7, first and third paragraph - It is not clear what is meant by "individual mean" scores. Are the scores provided the mean scores from 3 rabbits for erythema at the three different time points?

p.8-9, Discussion and Conclusion - The name of the ingredient in both of these sections needs to be corrected to "2-Amino-4-Hydroxyethylaminoanisole".

p.8, Discussion - Please revise the first paragraph. As 2-Amino-4-Hydroxyethylaminoanisole Sulfate is the ingredient being used in hair dye products, and it is the ingredient for which safety data are available the focus of the Discussion should be on the Sulfate salt not the free base. The term "biologic functions" is not appropriate as it suggests that this ingredient has a function in the body.

p.8 - The second paragraph of the Discussion appears to be boiler plate language that does not apply to this hair dye ingredient. The paragraph states that "irritation and sensitization data are not available in all cases". Irritation and sensitization data are available for 2-Amino-4-Hydroxyethylaminoanisole Sulfate. The Discussion should not imply that irritation and sensitization data are not available for this hair dye ingredient.

p.8, third paragraph - The information currently in the report on exposure to hair dye reaction products is very limited. Therefore, the third paragraph is not appropriate for the Discussion. The CIR Expert Panel should determine whether a "boiler plate" statement should be developed to

justify why the safety assessment focuses on the ingredient rather than reaction products that may form in a formulation containing this ingredient plus other oxidative hair dye ingredients and hydrogen peroxide.

p.8, last paragraph - As this is an oxidative hair dye ingredient, the following sentence should be deleted. "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."