BLUE

Safety Assessment of Hydroxypropyl Bis(*N*-Hydroxyethyl-*p*-Phenylenediamine) HCl as Used in Cosmetics

Status:	Draft Final Report for Panel Review
Release Date:	August 16, 2013
Panel Meeting Date:	September 9-10, 2013

The 2013 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; Ronald A Hill, 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 Lillian J. Gill, D.P.A. This report was prepared by Lillian C. Becker, Scientific Analyst/Writer.

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Cosmetic Ingredient Review

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MEMORANDUM

To: CIR Expert Panel and Liaisons

From: Lillian C. Becker, M.S.
Scientific Analyst and Writer

Date: August 16, 2013

Subject: Draft Final Report of Hydroxypropyl bis(N-Hydroxyethyl-p-Phenylenediamine) HCl As Used In Cosmetics

Attached, please find the Draft Final Report of Hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine) HCl. No new data have been submitted or discovered. Comments from industry have been addressed. There were no comments submitted from the public.

The Panel is to review the report on this hair dye and ensure that the Abstract, Discussion, and Conclusion reflect the Panel's thinking. The Panel should then issue a Final Report.

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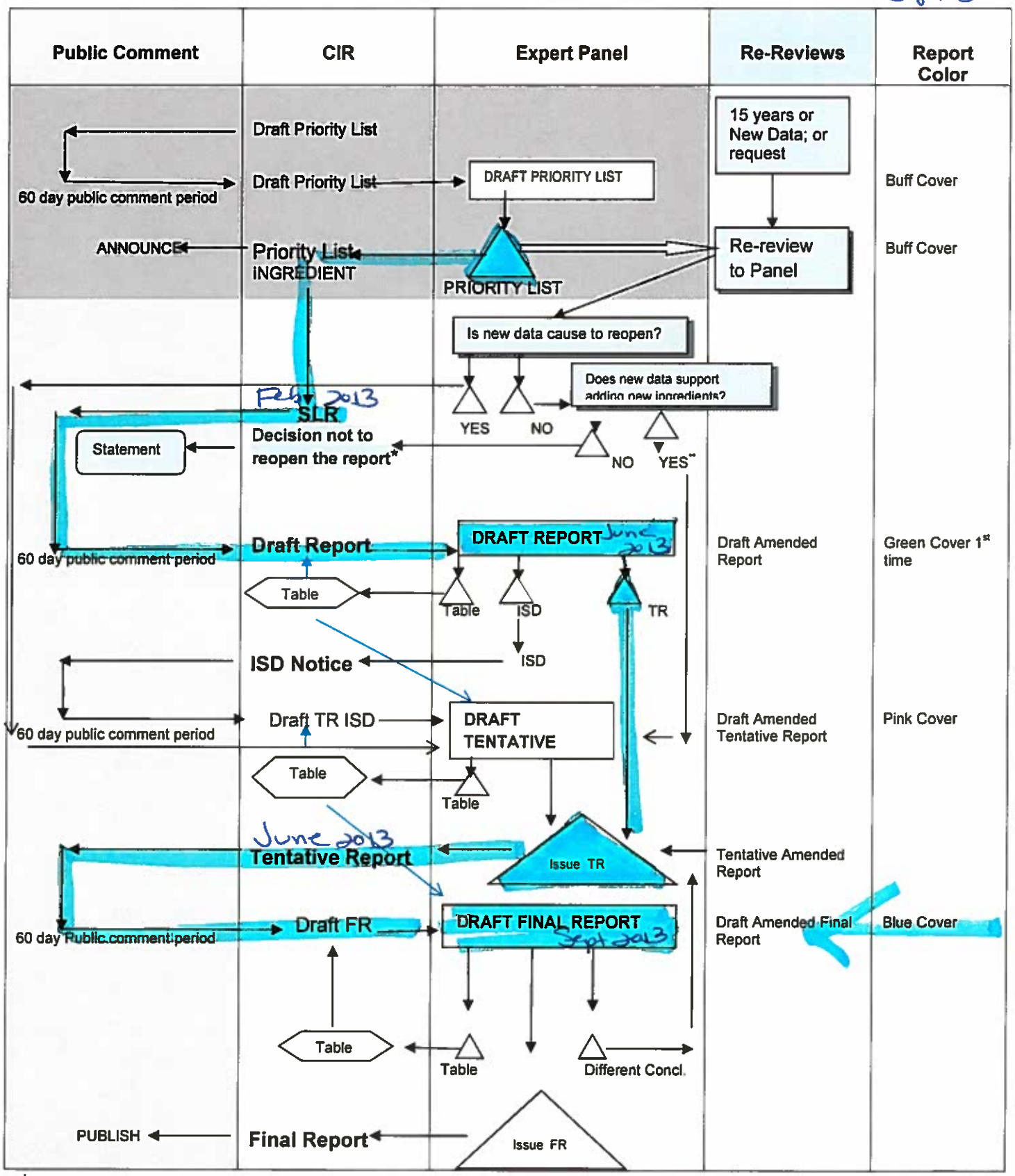
Hydroxypropyl Bis(N-Hydroxyethyl-p-Phenylenediamine) HCl

SAFETY ASSESSMENT FLOW CHART

Sept 2013



*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

History of Hydroxypropyl Bis(N-hydroxyethyl-p-phenylenediamine)HCl

February, 2013 – SLR was posted for public comment.

June, 2013 – Panel examined the Draft Report and issued a safe as used conclusion.

September, 2013 - The Panel examines the Draft Final Report and issues as Final Report.

Hydroxypropyl bis (N-hydroxyethel-p-phenylenediamine) HCl Data Profile for September, 20113 Writer - Lillian Becker																		
	ADME			Acute toxicity			Repeated dose toxicity			Irritation			Sensitization					
	Dermal Penetration	Log K_{ow}	Use	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Irritation	Dermal Irr. Animal	Dermal Irr Human	Sensitization Animal	Sensitization Human	Repro/Devel toxicity	Genotoxicity	Carcinogenicity	Phototoxicity
	X		X	X	X		X			X	X		X		X	X		X

Search Strategy for
Hydroxypropyl bis-(Hydroxyethyl-p-Phenylenediamine)HCl

Chemical name and CAS no. searched in SciFinder and the internet. Hits for SCCP and SCCS reports.

Minutes from June, 2013 for
Hydroxypropyl Bis(*N*-Hydroxyethyl–*p*-Phenylenediamine) HCl

Dr. Marks' Team

DR. MARKS: So this is the first time we've seen this hair dye ingredient. It's an oxidated dye. It's just one ingredient. You don't have to worry about add-ons or 110 hair dyes.

Ron, Tom, any needs? Do we move forward issuing a tentative report with "safe?"

DR. SHANK: Yes, go ahead.

DR. MARKS: Yep. Tom?

DR. SLAGA: Safe.

DR. MARKS: Ron Hill?

DR. HILL: Yes, I agree with that, but I was not happy with the terse language of the memo from the Hair Council at the end of this PDF, which is taking a second to come up, because they're talking about, again -- let's see, exposures to precursors and cutlers are low. And, again, I say Julie Skare gave us two presentations, a short one and a long one. We only had the slide set for the short one, but I have long notes for the long one. I agree if her people followed the instructions and only exposed the hair versus the -- try to be sure that they get the roots colors, and, therefore, expose the scalp, that's part of it.

But the other thing is, I remember, and I have notes on it from her long presentation, that the kinetics, and I hate to keep trying to support my credibility, but I studied chemical kinetics at the graduate level in two very robust courses, so I'm familiar with kinetics. Anyway, in some of these, because the rate of formation of the intermediate is slower than the oxidated -- the reactions that happen after that, the levels stay low. But that was not universally true for all the ones that they looked at, that there were some intermediates to build up to appreciable levels, and could potentially expose the scalp if somebody didn't follow the directions.

So in this particular ingredient, I have no concerns of safety. I have no concerns about any of those kinds of intermediates in this case. But I wasn't happy with the stance taken in that memo by the Hair -- excuse me -- Hair -- I got it going -- let me unrev --

SPEAKER: Hair Coloring --

DR. HILL: -- Hair Coloring Technical Committee, thank you, HCTC, because I think that they are either ignoring a good bit of the data that group with Julie Skare and were diligently developing where they did, in fact, measure appreciable concentrations of intermediates in some of the oxidative ingredients. I remember that very clearly. I mean, I caught the kinetics of all that. Some of them build up to appreciable concentrations. As long as somebody makes sure they're only exposing the hair and never get any on their scalp for any length of time, they still have no problem.

I know people who color their hair, and very often they want the roots to get colored, and so the scalp is going to get exposed to that. I'm not suggesting that we have a -- I mean, we already carcinogens in these ingredients, right? So but I just think that going forward, because the analytical technology exists, you know, if you're going to tell me that intermediate doesn't build up, prove it to me. You can do the kinetic studies in a way that's very compelling. And I realize I'm talking about the employment of analytic chemists who don't come cheap, but --

DR. MARKS: So would that change us issuing a tentative report?

DR. HILL: No. No, not at all. Not at all.

DR. MARKS: Okay.

DR. HILL: Not in this ingredient.

DR. MARKS: It looks like Belsito would be -- and then the only other thing, self-testing boiler plate. Is that

in there? Did I miss it?

MS. BECKER: It should be near the end. It's the first time you're seeing it where there's no discussion.

DR. MARKS: Okay. So obviously that'll need to go in. Okay. Any other comments?

DR. SHANK: Just a comment.

DR. MARKS: Oh, okay.

DR. SHANK: Table 1, it gives the color of this chemical, blue, white, gray, beige.

SPEAKER: All of them?

DR. SHANK: Yes. So are we dealing with a very unstable compound, or are we dealing with a mixture? Which color comes first? Why would it have four -- five different colors? Just a comment.

DR. HILL: Where are you --

DR. SHANK: A rhetorical -- Table 1 --

DR. SLAGA: I didn't catch that.

DR. SHANK: Fourteen, page 14.

DR. SLAGA: Blue, gray, white – I didn't look at that. Well, they're all different references.

DR. SHANK: Well, I mean, come on. You use a spectrometer of some sort, right?

DR. SLAGA: No, I totally agree with you. It seem impurity might --

DR. MARKS: Too bad impurities are in here.

DR. HILL: Impurities do vary because of the way these things are -- probably the analytics are generated from the nitro compounds. And depending on exactly how you do the reduction, then you get -- end up with -- because I've done those kind, and it's usually something like ethanol, or there's some other possibilities, depending on -- very, very low concentrations of an impurity can confer those kinds of colors to it.

DR. MARKS: Since this is a tentative report, Lillian, could you query industry and find out why they're different colors? I agree, that's intriguing.

MS. BECKER: Jay, can we query industry?

MR. ANSELL: Honest to goodness, I don't know the difference between white, gray, and beige to begin with. I'm not very good on my colors, but I'm not thinking these are actually different colors.

DR. HILL: The amounts of the nitro --

DR. MARKS: Blue or gray --

DR. HILL: -- blue. There's blue in there, too.

MS. BECKER: Yeah.

MR. ANSELL: Yeah, blue-gray, and white-gray, and beige.

MS. BECKER: Yeah, so there could be a difference between ecru and eggshell.

DR. MARKS: At any rate --

DR. SHANK: Okay, never mind.

DR. MARKS: -- it would be interesting. That's not going to hold it up obviously.

DR. HILL: Well, no, there's a good reason you asked that question, which is the compound itself is not intrinsically colored. I mean, it's going to -- so those colors are coming from low levels of impurities.

MS. FIUME: The rest --

DR. MARKS: Inquiring minds like Dr. Shank would like to know that. Okay. It's almost -- it's 10 of 3:00. Do we need to take a break, or do you want to trudge on? We have about --

MS. FIUME: The rest --

SPEAKER: Page 9.

MS. BECKER: Page 9 of the PDF.

MS. FIUME: Page 9 --

MS. BECKER: Dr. Marks, a question before you move on. Besides the hair dye boiler plate, is there anything else we want in the Discussion?

DR. MARKS: No. I mean, you have the epidemiology on the oxidation --

DR. HILL: Yeah, I see it.

MS. FIUME: Those are the detection limits, but it does say something.

DR. MARKS: Okay.

DR. HILL: I thought that wording was from the -- I flagged that actually that if they're below the detection limit, how do we know of their existence? So I think the answer is that they suspect they could be present, but then they were below the detection limit. The wording sounds funny, and I think I you can fix that.

You know, in compounds like nitroso and nitrose is not listed, but some of those could potentially could have color, but not most of these that are listed. It would be nitro compounds and some low amounts of polymers is how that will happen.

DR. MARKS: Okay. Shall we move on?

Dr. Belsito's Team

DR. BELSITO: Okay. Okay, so, then the next one is the hair dye, hydroxypropyl bis and hydroxyethyl para-phenaline, diamine, hydrochloride, and Lill's still with us and this is under hair dye, as well it should be.

So, anyway, first time we're looking at this. We've received an incorporated irritation and sensitization data, updated VCRP data and it's used in hair dyes, maximum.28 percent.

It's below the current SEC (inaudible) that gave a maximum ahead of.4 percent. The self-testing issue, where I think we just decided to make a comment about it, and that will keep abreast of what industry has to tell us about the risk versus benefit of suggesting people patch test themselves.

So, really are we ready to go ahead and I guess the only question that I had on this vis-à-vis the self-testing is that the data would suggest that this is not a sensitizer at the level it's used as opposed to paraphenylenediamine and some other oxidative hair dyes that are used, but it's exempt from Coal Tar Derivative

Act or whatever that specific act from 1930-something is because it's supposed to carry the label.

So, if we have a coal tar-type hair dye that doesn't cause sensitization, does it still have to carry that label?

DR. ANDERSEN: I mean, others can comment on this, but it becomes a manufacturer's choice. FDA offers the opportunity to be exempted from provisions of the Food, Drug, and Cosmetic Act if you add this label. If as a manufacturer you determine that you didn't need those exemptions, that your product was not going to be adulterated or misbranded, then you could presumably omit those statements. I don't think anybody would ever take that step. I think that wouldn't be good policy, but I think -- I don't know, Stan, if there's any reason that a manufacturer couldn't decide not to include it. I mean, it's not mandatory. You just get something if you do include it.

MR. MILSTEIN: That's true. It's only a safe harbor from the Section 601(a) adulteration provisions of the act. It's not to say that the agency couldn't determine that the product is not safe for use based on other criteria, but the safe harbor's there and it's available if the instructions for patch testing are given and if the exclusivity language warning is also presented on product package labeling.

DR. BELSITO: Thank you. Okay, comments from you guys so we can move on, safe as used and --

DR. LIEBLER: I had safe as used unless the dermatologists think we need HRIPT.

DR. BELSITO: Label it, use the safe harbor.

DR. SNYDER: What about the UV data? Did we --

DR. BELSITO: Page what?

DR. SNYDER: I'm looking right now. I'm trying to scroll down here, sorry.

DR. BERGFELD: Well, it has in the physical chemical properties range 200 to 400 nanometers absorption.

DR. SNYDER: Yes, so, a peak of 258, unless the well-defined peak of 302.

DR. LIEBLER: Well, the 258 wouldn't be a concern, but the 302, depending on how big it is --

DR. BERGFELD: But it's a wash-off. A rinse-off.

DR. SNYDER: Okay, I just had to bring that was all.

DR. BERGFELD: I had it highlighted, too.

DR. LIEBLER: So, on page 12 under "phototoxicity," it describes the experiment --

DR. BELSITO: Yes.

DR. LIEBLER: But it doesn't indicate the result. Oh, I'm sorry, it says it in the first sentence. I'm sorry, that did not cause phototoxicity or -- never mind.

DR. BELSITO: Yes.

DR. LIEBLER: So, just because you've got an absorbance band above 280, it means it's more possible, but no means certain that you could have problems.

DR. BELSITO: Yes. And the other issue here is that by the time the woman walks out of the salon, unless it was a really sloppy dye job and she got all this hair dye on her skin, there's not going to be any of that chemical left on her hair to create an issue.

DR. BERGFELD: That was my thought and I thought I had heard this that it's all consumed in the first few minutes anyway.

DR. LIEBLER: Correct.

DR. BELSITO: Exactly. And I should say men because there are some men who dye their hair, as well. That was rather sexist of me.

DR. BERGFELD: Yes. That's on the record.

(Laughter)

DR. ANDERSEN: Dan, apropos of the construct of this paragraph, Lill did a great job of capturing and getting the message that Kevin Fries has been trying to send about clear writing, put your message in the first sentence and then give the details. So, we'll see how this carries out, but Kevin is starting to have an impact.

DR. BERGFELD: That almost takes the place of the previous editor for the Journal of Toxicology, where you put the little summary statement first before the paragraph.

MS. BECKER: Besides the UV issue, anything else you want to include in the Discussion?

DR. BELSITO: No, I think that the usual hair dye discussion, I mean, taking verbatim, including labeling for testing and the whole issue that we're grappling with self-testing.

DR. BERGFELD: Would you discuss the phototoxicity when it's really quite negative in the guinea pig model and its (inaudible)? Would you even bother to discuss it?

DR. BELSITO: Well, I think we could say we noted that there was a small absorption peak in the UV range and that the data from phototoxicity and the fact that the product would be consumed gave the panel comfort or however you want to phrase it.

MS. BECKER: Thank you.

DR. BERGFELD: Nice job.

MS. BECKER: Thank you.

DAY TWO

DR. BERGFELD: Moving on, then, to the hair dye. Dr. Belsito?

DR. BELSITO: Okay, so this is the first time we're seeing this report on hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine hydrochloride. And basically, we thought this was a safe as used, and --

DR. MARKS: Second. (Laughter)

SPEAKER: He still wants to talk.

DR. BERGFELD: Go ahead, Don.

DR. BELSITO: No, that was it.

(Laughter)

DR. BERGFELD: Any discussion?

DR. BELSITO: Usual boilerplate and self-testing, and the little caveat that we'll continue to follow whether self-testing is right or wrong.

DR. BERGFELD: Is that going in the minutes or the document?

DR. BELSITO: It's going in the document, yes.

DR. BERGFELD: Okay, all right. Any other discussion? I'll call the question. All those in favor of safe in the hair dye?

(Motion approved by show of hands)

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ABSTRACT

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl is an oxidative hair dye. The CIR Expert Panel reviewed relevant animal and human data related to the ingredient. The Panel concluded that hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl was safe as a cosmetic ingredient in the practices of use and concentration of this safety assessment in cosmetics.

INTRODUCTION

This is a safety assessment of the oxidative hair dye hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl as used in hair dye products.

CHEMISTRY

Definition and Structure

The structure of hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (CAS no. 128729-28-2) is shown in Figure 1.

Physical and Chemical Properties

The physical and chemical properties are provided in Table 1. Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl is a substituted, dimeric aromatic amine salt. The $\log K_{ow}$ @ 20°C of this hair dye is -5.[1]

Ultraviolet light absorption was reported in the range of 200 – 400 nm (0.01 g/L in deionized water) with a peak at 258 nm.[2] There is a smaller peak at 302 nm. In the visible range of 350 – 800 nm (10 g/L in deionized water) there was a peak at 415.5 nm. There is a smaller peak at 570 nm.

Impurities

In studies submitted to the Scientific Committee on Consumer Products (SCCS), the purity of hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl was reported to range from 94.6% - 99.8%.[3] The following impurities were tested for and found to be below the detection limits: 2-phenylamino-ethanol (< 200 µg/g), 1,3-bis[(2-hydroxyethyl)-(4-nitrosophenyl)amino]propan-2-ol (< 100µg/g), and 1,3-bis[(2-hydroxyethyl)phenylamino]propan-2-ol (<100 µg/g).

USE

Cosmetic

Data on ingredient usage are provided to the Food and Drug Administration (FDA) Voluntary Cosmetic Registration Program (VCRP).[4] A survey was conducted by the Personal Care Products Council (Council) of the maximum use concentrations for ingredients in this group.[5] Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl was reported to be used in 75 hair dyes and colors at a maximum concentration of 0.28%.

Because this ingredient is only used in one category, no use table was developed.

While an earlier opinion of hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine)HCl stated that this hair dye may be used up to 3.0% (before mixing with hydrogen peroxide for application) so that the final concentration applied by the consumer does not exceed 1.5%, the current position of the Scientific Committee on consumer Products (SCCP) is that hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl is safe at a maximum concentration on the head of 0.4%.[3,6] European regulations state that after mixing under oxidative conditions the maximum concentration applied to hair must not exceed 0.4% (as tetrahydrochloride).[7]

Hair Dye Caution Statement – FDA Labeling

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl 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 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.

Thyssen et al.[8] concluded, however, that, in its present form, the hair dye self-test has severe limitations. The authors warned that, if the use of a hair dye self-test to predict contact sensitization becomes widespread, there is severe risk that a tool has been marketed that may cause morbidity in European consumers. In an accompanying editorial, An Goossens,

on behalf of the European Society of Contact Dermatitis, asserted that industry is focusing on predicting the risks from exposure to hair dyes by having millions of European consumers perform a self-test prior to each hair dying and stated that it is the opinion of the ESCD that attention must be given to reducing the risks of serious allergic reactions by improving the safety of the products themselves.[9]

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

Dermal/Percutaneous

^{14}C-Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (25 mg/kg in water) administered to the clipped skin of Wistar Hans rats for 30 min was primarily recovered in the application site wash and the dressing (males, 94.2 ± 3.91%; females, 96.86 ± 2.96%).[10] Recovery in urine and feces was < 1%. Recovery in the skin (dermis and epidermis) was < 0.2%. Of the small amount that was absorbed, most of the radio activity was eliminated in the feces (> 80%) within 72 h. There were no gender differences in the results.

When applied to human skin for 30 min in a diffusion cell, < 0.2% of the radioactivity of a hair dye product (20 mg/cm^2) containing ^{14}C radio labeled hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (3.67 ± 0.25%) mixed with either hydrogen peroxide or water was recovered in the receptor cell.[11] The receptor fluids were sampled at 0, 0.5, and 1 h then every hour up to 24 h. Most of the dye was recovered from the skin surface (hydrogen peroxide, 93.9 ± 2.7%; water, 98.2 ± 4.0%). The stratum corneum contained 1.78 ± 0.87% and 1.32 ± 0.96% of the dye and the epidermis/dermis contained 0.55 ± 0.33% and 1.85 ± 1.68%, respectively.

When applied to human skin for 30 min in a diffusion cell, ~0.01% of a ^{14}C radio-labeled hair dye (14.0 µL in water; ~20 mg/cm^2) containing hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (0.34%, 0.8%) mixed with either hydrogen peroxide or water was recovered in the receptor cell.[1] Samples were collected from the receptor cell at 0.5 h and 1 h, then hourly after that. Most of the dye was recovered from the skin surface (hydrogen peroxide, 98.31 ± 2.68%; water, 98.72 ± 2.27%). The epidermis/dermis contained 0.90 ± 0.92% and 0.80 ± 0.77% of the applied dye, respectively.

When applied to heat separated human abdominal or breast epidermis in a Franz diffusion cell (n = 7), 0.004% (in the absence of hair) and 0.005% (in the presence of hair) of the administered dose was present in the receptor cell.[3] Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (3.3% in a formulation containing 0.64% p-aminophenol and then mixed 1:1 with hydrogen peroxide for a final concentration of 1.65%; 40 mg) was applied to the skin for 30 min, with or without finely chopped bleached hair. Samples were measured by high performance liquid chromotography. (HPLC).

Oral

When ^{14}C-hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl was orally administered to Wistar Han rats, the mean plasma total radioactivity levels increased from time 0 until the c_{max} (1558 ± 157 ng-eq/g for males and 1678 ± 540 ng-eq/g for females) at 1 h (males) or 2 h (females), and then decreased until the last quantifiable time points at 6 h (281 ± 15 ng-eq/g) or 8 h (224 ±53 ng-eq/g), respectively.[12] Blood samples (n = 3/sex/time point) were collected at 0, 1, 2, 4, 6, 8, 24, 48, and 72 h after treatment.

Other rats (n = 3/sex) were weighed and urine/feces/cage wash collected for 0 - 6 h and 6 – 24 h then every 24 h up to 168 h. Following oral gavage of the isotope-labelled mixture at 100 mg/kg, the mean total cumulative excretion of the radioactive dose in the summed excreta over the 168-h period was 98.3 ± 2.7% and 96.3 ± 3.4% for the males and females, respectively. A mean of 2.5 ± 0.3% and 95.4 ± 2.5% of the test substance was eliminated in the urine and feces, respectively, for the males and 3.7 ± 0.3% and 88.6 ± 9.3% for the females. The cage contained < 5% of the test substance for both sexes. Most of the radioactivity (90.7% and 72.9 %, respectively) was eliminated in the summed urine and feces within 24 h, > 95% of which was in the feces.[12]

TOXICOLOGICAL STUDIES

Acute Toxicity

Dermal – Non-Human

The dermal LD_{50} for hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl was > 2000 mg/kg (the maximum dose tested) for Sprague-Dawley rats (n = 5/sex).[13] One rat had a slight decrease in spontaneous activity at 4 and 6 h after treatment.

Oral – Non-Human

The oral LD_{50} of hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl was reported to be 2186 mg/kg (95% C.I. 1797-2965) for Sprague-Dawley rats (n = 5 females, 5/sex for 2000 mg/kg group).[14] The fasted rats were administered the test substance (1100, 1600, 2000, 2600 mg/kg in water; 10 ml/kg). No deaths occurred in the 1100 and 1600 mg/kg female groups. In the 2000 mg/kg group, 2/5 females and 3/5 males died. In the 2600 mg/kg group, 4/5 females died. Except for 2 animals which died on day 3, all deaths occurred within 30 minutes of treatment. Hypoactivity, sedation,

piloerection and dyspnea were observed in both sexes. Males exhibited lateral decubitus. The first signs were observed at 30 min after treatment. For those that did not die, recovery was complete on day 7 for the females and day 5 for the males.

Wistar HanIbm:WIST (SPF) rats (n = 2) exhibited a reduction of spontaneous activity eyelid closure, and apathy when orally administered 1500 mg/kg hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl.[15] At 2000 mg/kg, there was a reduction of spontaneous activity eyelid closure, apathy, abdominal position, and one death observed.

Repeated Dose Toxicity

Oral – Non-Human

In a range finding study where Sprague-Dawley rats (n not provided) were administered hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (50, 200, 800 mg/kg/d) for 2 weeks, the rats in the high-dose group had a slight decrease in body weight gains, serum glucose, and total protein levels.[16,17] A dose of 800 mg/kg/d resulted in: ptyalism and signs of poor clinical condition in both sexes, slightly lower body weight gain in males (-11% compared to controls), lower serum glucose (-26%) and higher triglyceride (x 1.5) levels in males, and in the kidneys, minimal to slight brownish pigment in the tubular epithelium and slightly higher incidence and severity of tubular dilatation in both sexes. The mid-dose group had a slight decrease in serum glucose levels. There were no effects observed in the low dose group.

The oral NOAEL for hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine)HCl was 25 mg/kg/d when administered to rats for 13 weeks.[17] The test substance (25, 100, 400 mg/kg/d in water) was administered to Sprague-Dawley rats (n = 10/sex) by gavage; the rats were then killed and necropsied. There were no clinical signs in the low-dose group.

There was ptyalism, loud breathing, and/or regurgitation in the mid- and high-dose groups from week 4. Pink urine, brown-colored tails, and brown or black feces were also observed in these groups. One male from each of the mid- and high-dose groups died; aspiration pneumonia due to regurgitation was considered a contributing factor. Body weights and feed consumption were similar to controls. Opacification of the lens was observed in one female in the high-dose group. Females in the high-dose group had higher activated partial thromboplastin time and higher serum urea and creatinine levels were observed in females in the mid and high dose groups. Urinalysis and macroscopic examination of tissues were unremarkable. Microscopic examination revealed tubular basophilia in the kidneys of the males in the high-dose group and many of the organs and tissues had a brownish pigmentation, probably due to the color of the test material.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

The NOAEL for reproductive and developmental toxicity was > 800 mg/kg/d for Crl CD (SD) BR Sprague-Dawley rats (n = 25) orally administered hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (50, 200, 800 mg/kg/d in water on days 6 – 15 of pregnancy).[16] On day 20, the dams were killed and necropsied. Other than colored urine in one dam in the low dose group and all the dams in the mid and high dose groups, there were no clinical signs. The necropsies were unremarkable. The mean number of corpora lutea, implantation sites, post-implantation loss, live fetuses, sex ratio, and fetal body weights were similar to controls. There were no treatment related anomalies in the fetuses.

GENOTOXICITY

In Vitro

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (312.5, 625, 1250, 2500, 5000 µg/plate with metabolic activation; 62.5, 125, 250, 500, 1000 µg/plate without) was not mutagenic to *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537) and *Escherichia coli* (strain WP2uvrA) except for weak mutagenic activity observed (2.2 fold increase in revertant colonies) at 5000 µg/plate with metabolic action in the TA100 strain.[18] The test with metabolic activation was repeated except for the highest dose (125, 250, 500, 1000, 2000 µg/plate) with the same result. The test without metabolic activation was repeated at the same concentrations with the same result as the first assay.

In a mammalian cytogenetic assay using Chinese hamster ovary (CHO) cells, hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (30, 100, 300, 1000, 3000, 5000 µg/mL with metabolic activation; 12.5, 25, 50, 100, 150 µg/mL without) did not induce an increase in aberrant cell frequency with metabolic activation at any exposure, except for 100 µg/mL without metabolic activation.[19]

When this assay was repeated (125, 250, 500, 750, 1000 µg/mL with metabolic activation; 12.5, 25, 50, 75, 100 µg/mL without), the test substance did not induce an increase in aberrant cell frequency with metabolic activation.[19] However, without metabolic activation, the test substance increased the instances of cells with structural chromosome aberrations at 75 µg/mL. In each case, these positive findings in the without metabolic activation group were not observed at a higher dose level. The authors suggested that the finding were not relevant to assessing a dose-response.

In Vivo

In an unscheduled DNA synthesis (UDS) assay of hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (150, 1500 mg/kg in distilled water; 10 ml/kg) using Wistar HanIbm:WIST (SPF) rats (n = 4), there was no induction of UDS in the hepatocytes of the treated rats.[15] The hepatic samples were collected at 2 h (1500 mg/kg) and 16 h (150, 1500 mg/kg) after the rats were administered a single oral dose of the test substance. The hepatocytes were cultured and the cells examined for UDS.

In a micronucleus test, hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (375, 750, 1500 mg/kg/d) orally administered for 2 days to Swiss OF1 mice (n = 5/sex) did not induce damage to the chromosomes or the mitotic apparatus of the bone marrow cells of the mice.[20]

In a micronucleus test, hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (500, 1000, 2000 mg/kg/d) orally administered for 2 days to Sprague-Dawley rats (n = 5/sex) did not induce damage to the chromosomes or the mitotic apparatus of the bone marrow cells of the mice.[21]

CARCINOGENICITY

No published carcinogenicity studies were discovered and no unpublished data were submitted.

IRRITATION AND SENSITIZATION

Irritation

Dermal – Non-Human

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (10% in purified water; 0.05 ml) was not irritating in a repeated application irritation test using Dunkin-Hartley guinea pigs (n = 3/sex).[22] The test substance was administered to the clipped skin daily for 14 days. The guinea pigs were killed and the test site examined microscopically. There were no clinical signs. There was a very slight erythema on all guinea pigs on day 9 and two on days 10 and 15. Almost all of the animals had dry skin at the test site. There was a slight black coloration of the skin starting on days 3 and 4 that could have masked very slight erythema.

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (5% in distilled water; 0.05 ml) was not irritating in a patch test using New Zealand White rabbits (n = 3).[23] The test substance was administered to clipped skin under semiocclusion for 4 h and observed at 1, 24, 48, and 72 h and then daily up to day 9. No skin reactions were observed in one rabbit. Very slight or well-defined erythema was observed at 24 or 72 h after treatment in the other two rabbits. No edema was observed. There was dryness of the skin observed on days 5 - 8 in one rabbit. Mean scores over 24, 48 and 72 h for each animal were 0.0, 1.7, and 0.3 out of 4 for erythema and 0.0 for edema.

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (100% dampened with water; 500 mg) was an irritant to male New Zealand White rabbits.[24] The test substance was administered to clipped skin under occlusion for 3 min (n = 1), 1 h (n = 1), and 4 h (n = 3). After 3 min, erythema (masked by a black coloration of the test site) was observed and persisted up to day 10. Slight edema was noted 1 h after removal of the dressing. After 1 h, slight to severe erythema was observed on days 1- 11. Severe to slight edema was observed on days 1 - 6. After 4 h, erythema (masked by a black coloration of the test site) persisted up to day 15. Slight to severe edema was observed on days 1 - 5 in two rabbits. The third rabbit had slight edema 1 h after removal of the dressing. The mean scores over 24, 48 and 72 h for individual rabbits were 0.0, 2.7 and 3.3 out a possible 4 for edema. Due to the skin coloration, erythema could not be scored.

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (0.5 g in 0.5 mL distilled water) had a primary irritation index of 3.4 when administered to the intact and abraded clipped skin of New Zealand White rabbits (n = 3).[25] Slight to well-defined erythema and slight to severe edema were observed.

Ocular

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (100%; 0.1 mL) caused opalescent corneal opacity, iridial inflammation, and severe conjuctival irritation as well as sloughing of the cornea, hemorrhage, and a pale appearance of the nictitating membrane when administered to the eye of one New Zealand White rabbit.[26]

In a repeat of the above experiment (100 mg; n = 1), the test material caused severe ocular reactions including severe to marked chemosis, slight to moderate conjunctival redness, iris lesions, and moderate to marked corneal opacity. Neovascularisation of the cornea was observed at 72 hours.[3]

Hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine) HCl (5% in water; 0.1 mL) was not an ocular irritant to New Zealand White rabbits (n =3).[27] The eyes were not rinsed and were observed at 1, 24, 48, and 72 h after administration.

Sensitization

Dermal – Non-Human

In a Buehler test, hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (50% in distilled water; 0.5 mL) administered to the clipped skin of Dunkin-Hartley guinea pigs (n = 10/sex) did not induce sensitization when challenged (5% and 20%).[28] During the induction period, very slight to slight cutaneous reactions were observed in 8/20 guinea pigs.

In a guinea pig maximization assay using Dunkin-Hartley guinea pigs (n = 10), hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (0.1% in a sterile isotonic aqueous NaCl solution; 0.5 mL) administered by subcutaneous injections did not induce sensitization when challenged at 25% administered in a dermal patch.[29]

In a Magnusson-Kligman maximization test using Dunkin-Hartley guinea pigs (n = 10/sex), hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (1% in sterile isotonic saline) administered by intradermal injections and challenged at 50% in a dermal patch was classified as a sensitizer.[30] At 24 h, very slight, well-defined, and marked erythema were

observed in 2/20, 11/20 and 7/20 guinea pigs, respectively. There was also slight edema observed in 11 guinea pigs and severe edema in one animal. Dryness of the skin was observed in 9/20 guinea pigs. Very slight black coloration of the skin was observed in 3 guinea pigs. At 48 h, very slight, well-defined, marked, and severe erythema were noted in 1/20, 4/20, 1/20 and 2/20 guinea pigs, respectively. Crust formation was observed in 3 guinea pigs. Dryness of the skin was observed in 14/20 guinea pigs. The dryness was severe enough to mask the evaluation of erythema in 5/20 treatment sites. Very slight to slight black coloration of the skin was observed in 5 guinea pigs. The very slight erythema which did not persist at the 48-h reading in two guinea pigs was attributed to a possible slight irritant reaction. All of the other skin lesions were attributed to a sensitization effect.

Phototoxicity

Dermal administration of hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl (10% in purified water; 0.2 mL) to Dunkin Hartley guinea pigs (n = 10) did not cause phototoxicity or photosensitization when exposed to UVA or UVB lamp light.[31] For the phototoxicity assay, the test substance was gently massaged into the shaved backs of the guinea pigs and 30 min later they were irradiated by UVB (312 nm) then UVA (365 nm). For the photosensitization assay, the guinea pigs were administered the test substance and irradiated 6 more times. After a 20-day rest, the test substance was administered and the test sites were irradiated again (left flank UVA, right flank UVB). The test sites were scored for reactions at 1, 6, 24, and 48 h after application.

HAIR DYE EPIDEMIOLOGY

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl is an oxidative hair dye ingredient. 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. Currently available epidemiology studies provided insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. A detailed summary of the available hair dye epidemiology data is available at http://www.cir-safety.org/cir-findings.

SUMMARY

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl is an oxidative hair dye used in 75 hair dyes and colors at a maximum concentration of 0.28%.

Europe has established a 0.4% limit on hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl when used as an oxidative hair dye.

Less than 1% of the radio activity of [^{14}C]hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl was recovered in the urine and feces when dermally applied to the skin of rats. Less than 0.2% of the dye in water or hydrogen peroxide was recovered in the receptor cells using human skin. Most of orally administered test substance was eliminated through urine and feces and cleared from the blood of rats within 6 – 8 h.

The dermal LD_{50} was > 2000 mg/kg for rats. The oral LD_{50} for rats was 2186 mg/kg.

The oral NOAEL for hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl was 25 mg/kg/d for 13 weeks for rats.

The NOAEL for reproductive and developmental toxicity was > 800 mg/kg/d orally administered to rats on gestation days 6 – 15.

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl was not genotoxic to *S. typhimurium* and *E. coli* in an Ames test and a mammalian cytogenetic assay using CHO cells. The test substance was not genotoxic in an unscheduled DNA synthesis assay and two micronucleus tests.

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl was not irritating to guinea pigs up to 10% and rabbits up to 5%. It was severely irritating to rabbits at 100%.

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl was a severe ocular irritant to rabbits at 100% but was not an irritant at 5%.

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl was not sensitizing to guinea pigs up to 50% when applied dermally. However, when applied by intradermal injection, the test substance was sensitizing at 1%.

Hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl was not phototoxic at 10% when exposed to either UVA or UVB light. The hair day was not photosensitizing.

Currently available epidemiology studies provided insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers.

DISCUSSION

The Panel noted the minimal penetration of hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl through the skin and no toxicity was found at levels used in hair dyes. There was no genotoxicity demonstrated and this ingredient was not irritating or sensitizing.

The Panel noted that even though there was a small peak in the UVB range in the absorbance spectrum of this ingredient, there was no phototoxicity and little photosensitization demonstrated.

The Panel noted that the use of oxidative hair dye formulations involves exposure to precursors and coupling agents as well as to their reaction products. While reaction intermediates may be formed, human exposure is to the precursors and coupling agents and to reaction products, not to reaction intermediates. The exposures to the precursors and couplers are low (they are consumed in the color forming reaction), and the exposures to reaction products are even lower (they are adsorbed into the hair shaft itself and physically retained there). Therefore, safety assessments of oxidative hair dyes are driven by the toxicological evaluation of the ingredients (i.e. precursors and coupling agents), more than by the reaction products formed during use, and not at all by reaction intermediates.

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 toxicological endpoints, based on lack of strength of the associations and inconsistency of findings.

The Expert Panel recognized that hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl functions as hair dye ingredients and 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, 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. 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.

CONCLUSION

The CIR Expert Panel concluded that hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl is safe in the present practices of use and concentration described in this safety assessment in cosmetics.

TABLES AND FIGURES

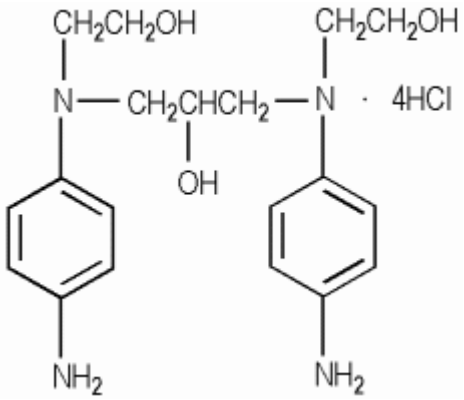

Figure 1. Chemical structure of hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl.

Table 1. Physical and chemical properties of hydroxypropyl bis(*N*-hydroxyethyl-*p*-phenylenediamine) HCl.

Property	Value	Reference
Physical Form	Powder	26
Color	Blue-grey	26
	White-grey	1
	Beige	13
Odor	Strong, irritating	2,3
Molecular Weight g/mol	506.3	1
Water Solubility g/L	760	1
Other Solubility g/L @ 22°C		
Ethanol	< 1	2
DMSO	≥ 20	2
log K_{ow} @ 20 °C	-5	1

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Lillian Gill, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

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

DATE: July 12, 2013

SUBJECT: Comments on the Tentative Report on Hydroxypropyl bis(N-Hydroxyethyl-p-Phenylenediamine) HCl

p.4, Abstract, p.9, Conclusion - The language used for the conclusion should be the same in the Abstract and Conclusion sections. The sentence should not end with "of this safety assessment".

p.4 - The acronym SCCS needs to be defined the first time it is used. As indicated in the references, the first opinion was from the Scientific Committee on Consumer Products (SCCP). The name of the committee was changed, and the second opinion was from the Scientific Committee on Consumer Safety (SCCS). This is not clear in the Cosmetic Use section.

p.4 - Please correct: "provisions of the of the Federal Food, Drug, and Cosmetic Act."

p.5 - In reference 10, did the investigators really measure the "test substance" or did they just measure radioactivity?

p.5 - Please revise the following as it is not clear what was studied in reference 11 or reference 1: "of a ^{14}C radio labeled hair dye containing hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine) HCl". What was labeled?

p.5 - How was Hydroxypropyl Bis(N-Hydoxyethyl-p-Phenylenediamine) HCl measured in reference 3?

p.5 - Please indicate the type of confidence interval 1797-2965 represents, e.g., 95% C.I.

p.6 - Because the concentrations tested in the two trials (with metabolic activation) of the Ames assay of Hydroxypropyl Bis(N-Hydoxyethyl-p-Phenylenediamine) HCl (reference 18) were not the same, it is not clear how the results of the second trial can be the same as the first trial. In the first trial a weak response 5000 µg/plate with metabolic activation was observed. In the second trial, 2000 µg/plate was the highest dose tested with metabolic activation. Were all doses in the second trial with metabolic activation negative?

p.7 - Please correct: "bone marrows cells" (this occurs twice). Please include the route of exposure used in the two micronucleus tests.

p.8 - Did the kinetic study in rats really measure the recovery of Hydroxypropyl Bis(N-Hydoxyethyl-p-Phenylenediamine) HCl or just the recovery of radioactivity?

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p.8 - In the Summary, please include the route of exposure for the 13-week study. The Summary should also note that Hydroxypropyl Bis(N-Hydoxyethyl-p-Phenylenediamine) HCl was not photosensitizing.

p.8 - In the Discussion, it is misleading to state that Hydroxypropyl Bis(N-Hydoxyethyl-p-Phenylenediamine) HCl is not sensitizing. One guinea pig study in which the animals were treated with intradermal injections with 1% followed by challenge with 50% was positive. Two other guinea pig studies were negative.