Safety Assessment of Basic Red 76 as Used in Cosmetics

Status:	Draft Final Report for Panel Review
Release Date:	May 10, 2019
Panel Meeting Date:	June 6-7, 2019

The 2019 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 Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Priya Cherian, Scientific Analyst/Writer.

1620 L Street, NW, Suite 1200 ◊ Washington, DC 20036-4702 ◊ ph 202.331.0651 ◊ fax 202.331.0088 ◊
cirinfo@cir-safety.org

Cosmetic Ingredient Review

Commitment & Credibility since 1976

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Priya Cherian, Scientific Analyst/Writer
Date: May 10, 2019
Subject: Safety Assessment of Basic Red 76 as Used in Cosmetics

Enclosed is the Draft Final Report on the Safety Assessment of Basic Red 76 as Used in Cosmetics (identified as *basred062019rep* in the report package). At the December 2018 meeting, the Panel issued a Tentative Report with the conclusion that this ingredient is safe in cosmetics in the present practices of use and concentration described in the safety assessment.

At the time the Tentative Report was issued, Basic Red 76, which according to the *Dictionary* is reported to function as a hair colorant and hair-conditioning agent, only had use in hair coloring formulations. However, according to 2019 VCRP data, this ingredient is also used in nail polish and enamel; concentration of use data were not reported by industry for this use. The Panel should determine whether the data in the report supports this use, and if it does, formulate language for addition to the Discussion. If the data do not support this use, and additional data are needed to determine safety for this use, then an Insufficient Data Announcement (IDA) should be issued to identify those data needs.

Comments received from the Personal Care Products Council (Council) (*basred062019pcpc_1* and *basred062019pcpc_2*) were received, and have been addressed.

The following are also included in this package for your review:

basred062019min: minutes from the December 2018 meeting

basred062019flow: report flowchart

basred062019hist: history

basred062019prof: data profile

basred062019strat: search strategy

basred062019FDA: 2019 VCRP data (US FDA)

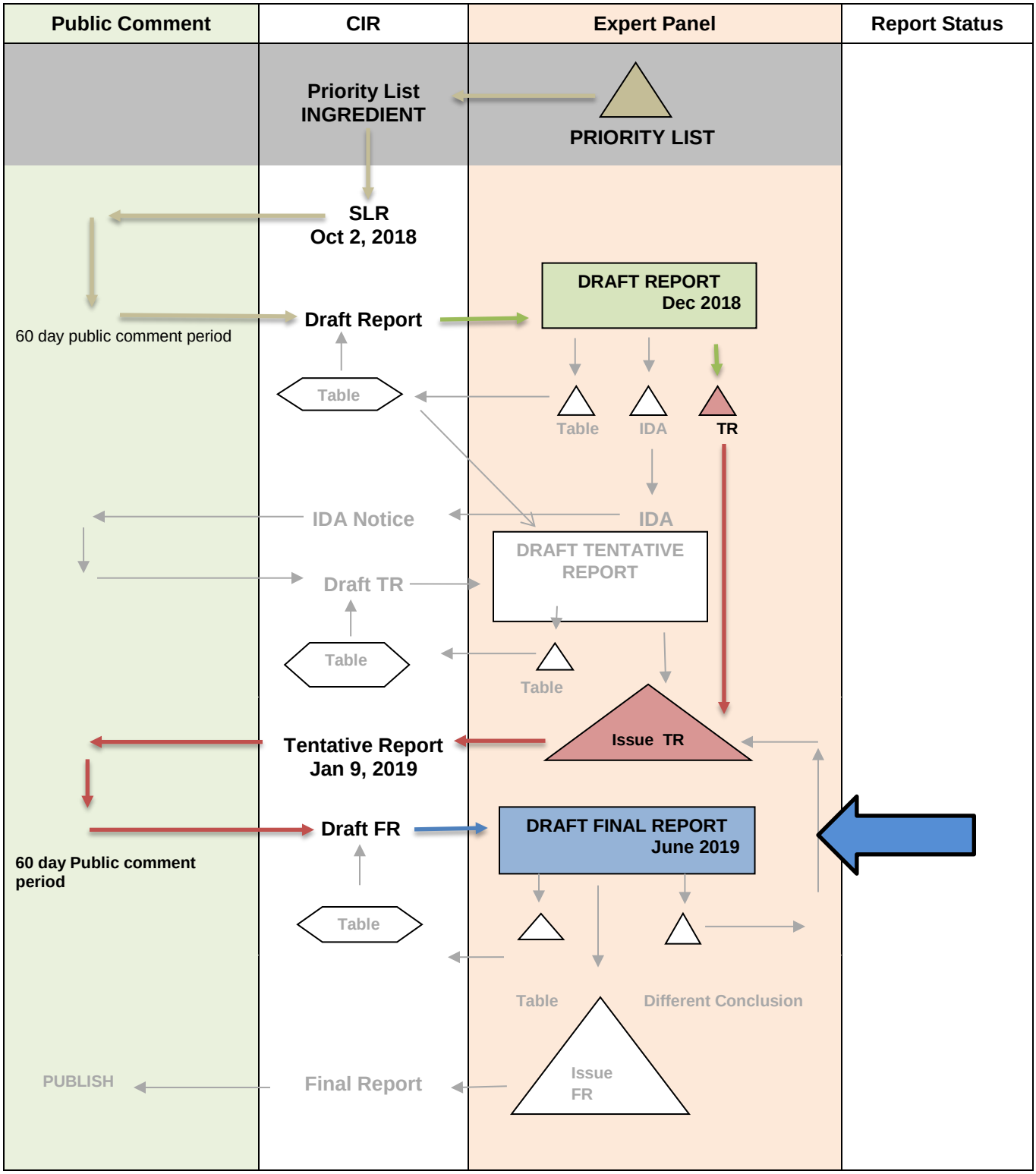
The Panel should carefully review the Abstract, Discussion, and Conclusion of this safety assessment. If these are satisfactory, and the new use type reported in the VCRP does not affect the conclusion, then the Panel should issue a Final Report. If additional data are required, the Panel should be prepared to identify those needs and issue an IDA.

1620 L Street, NW, Suite 1200 ◇ Washington, DC 20036-4702
(Main) 202-331-0651 (Fax) 202-331-0088
(Email) cirinfo@cir-safety.org (Website) www.cir-safety.org

SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Basic Red 76

MEETING June 2019



Basic Red 76 History

October 2, 2018: SLR posting

December 2018: Panel reviewed the Draft Report and issued a Tentative Report

January 2019: Council comments were received; VCRP data updated

June 2019: Panel reviews the Draft Final Report

Basic Red 76 Data Profile for ***June, 2019***. Writer – Priya Cherian																					
		ADME		Acute toxicity			Repeated dose toxicity			Irritation					Sensitization						
	Use	Log K_{ow}	Dermal Penetration	Oral	Dermal	Inhale	Oral	Dermal	Inhale	Ocular Animal	Ocular In Vitro	Dermal Animal	Dermal Human	Dermal In Vitro	Animal	Human	In Vitro	Repro/Devel	Genotoxicity	Carcinogenicity	Phototoxicity
Basic Red 76	x	x	x	x			x			x		x			x			x	x		

[Basic Red 76 – for June 2019 Panel meeting]

Ingredient	CAS #	InfoBase	SciFinder	PubMed	TOXNET	FDA	EU	ECHA	IUCLID	SIDS	HPVIS	NICNAS	NTIS	NTP	WHO	FAO	ECET -OC	Web
Basic Red 76	68391-30-0	1	No	No	No	Yes	No	No	No	No	No	1/1	No	No	No	No	No	Yes

Search Strategy

[*document search strategy used for SciFinder, PubMed, and Toxnet*]

[*identify total # of hits /# hits that were useful or examined for usefulness*]

Key words:

Basic Red 76; Basic Red; monoazo dye; 68391-30-0; hair dye toxicity

Basic Red 76 Minutes

December 2018 Meeting

Dr. Marks Team

DR. MARKS: Okay. The next one is Basic Red 76. We had one is hair dye ingredient, and that is in here. And this is the first review of this ingredient. It's a hair color and hair conditioning agent used for highlighting. Tom, Ron and Ron, comments? Obviously, carcinogenicity is always an issue with these hair dyes.

DR. SLAGA: Well, there's plenty of toxicity, irritation, sensitization, which are okay. Not positive. Genotoxicity is negative, both in bacteria and mammalian studies. And I don't believe there was a carcinogenicity in this case. I would say it's safe.

DR. MARKS: Yeah. I think that was page -- I have carcinogenicity, Page 12, with a question mark, Tom. And they say it -- oh, I know what it was. Potential metabolites of Basic Red 76, such as o-anisidine, have induced a number of multi-organ tumors. So, I guess we need a comment about that in the discussion.

DR. SLAGA: Yes.

DR. HILL: What's interesting about that is that, in general, human beings, our own physiology and cells

don’t do a whole lot of reductive metabolism of the diazo moieties, they tend to stay intact. But microbes do that; and our gut microbes do that. That’s classic prodrug stuff. Then, there were some issues that need to be cleaned up with the writing in here, concerning that. But anyway --

DR. SHANK: That can be all handled in the discussion.

DR. HILL: Well, no, it relates to if you just do an Ames test on the parent subject. The question is whether you’re going to -- again, fundamentally, we know hair dyes are a special exception case. But if you just do it on the parent compound, under circumstances where we don’t know if the microbes are reducing it to generate these other metabolites or not -- which will happen in human being, but I don’t know if it happens with bacteria in the scalp, it definitely happens in the gut.

I’m not raising a concern, really; it’s just a general issue with the way this is written, and what we do know, and what we don’t know. Which I’m not sure the Ames test gives the full picture, but I don’t know, because I don’t know, does scalp bacteria reduce these things,

because we don't. So, it's just used in -- you don't leave this on the hair other than to the extent the hair is dyed. After that, you rinse it and wash it, right?

DR. SHANK: Right.

DR. HILL: Okay, so --

DR. SHANK: The carcinogen is present at a very low concentration in the dye.

DR. HILL: Yeah. Yeah.

DR. SHANK: It's applied to the hair and rinsed off. So, potential for carcinogenicity, using the hair dye from that compound, is very, very low.

DR. MARKS: Now, the federal law, or regulation, is to pretest this, correct?

DR. ANSELL: To label.

DR. MARKS: According to label. I was interested in Page 9, where we're talking about this allergy alert. Should there be so much emphasis placed on this allergy alert, or we gonna put this in all? That's Page 9. It's the second paragraph above the toxicokinetic studies. Where it says, "furthermore, according to a report published in 2018, a different method of patch-testing was suggested."

DR. SHANK: Do we put that in all of them, though?

DR. MARKS: Yeah. Is that gonna go on all of them, it's just a precedent. And quite frankly, I could say, I'm not sure I would go into all the details. Just say that -- I mean, the biggest concern was, to me, the paragraph before. "In 2012, a report was published regarding such self-testing for contact sensitization to hair dyes. These authors concluded that, in the present form, hair dye self-test has severe limitations." So, we kind of go into --

DR. ANSELL: I think we have a boilerplate where there's an opportunity to discuss ongoing research in the area of hair dyes. If this is considered substantive, it might be addressed there. But to dump it in the middle of a Red 76 report, I agree, it seems odd. We're not making any recommendations or suggestions or --

DR. MARKS: I can see referencing them; but then they spend a lot of time on it to make -- to tell you the truth, I wasn't all that impressed with the allergy --

DR. ANSELL: What?

DR. MARKS: That test. I mean, essentially, it's a use test. The allergy alert -- nice name. I think if I

were to market it, allergy alert test sounds really good to get UAT. So, that was, Jay, sort of my concern. Do you put this much emphasis? And if you do, then is it going to be repeated for every hair dye going forward?

DR. ANSELL: Well, yes.

DR. SHANK: I thought it was sort of a boilerplate.

DR. ANSELL: That presupposes the that we agree that this was better.

DR. MARKS: Right.

DR. SHANK: No, it doesn't say we agree.

DR. ANSELL: It doesn't say anything.

DR. SHANK: It just says --

DR. ANSELL: Here's a publisher.

DR. SHANK: Well, there has been an alternative proposed -- put forward.

DR. ANSELL: Yeah. I think we have a place to discuss that stuff if we believe that this is elevated to that level. But no, not with the Red 76 report. I agree.

DR. MARKS: Maybe this could be shortened. Obviously, we should acknowledge it. It's in the literature, but just shortened and reference it, basically.

There are alternative testing methods to the present. And then the issue of sensitization from the testing itself. I don't think, again -- and point of fact, in real life, this is uncommonly done, prior to hair.

DR. SHANK: You could have just the first two sentences saying this has been developed; and then don't give the protocol.

DR. MARKS: Yeah. Exactly.

MS. LORETZ: Yeah. This is just the work that was given to you guys, on a study, to try to make sure that people are using the same conditions, and trying to optimize the conditions for it. It kind of reads differently here. It looks like it's new and different, but it was just trying to optimize, as you said, to see if it works. So, yeah. I think a sentence reference to it is great, but the protocol seems excessive.

DR. HILL: First two sentences work for me, from what you said.

DR. MARKS: So, we'll shorten it. Yeah. Good. Okay. Priya, you have that. I don't think I need to mention that tomorrow, unless -- what do you think, team? Do I need to mention that when we do this discussion? That

we've shortened the patch-testing section at Page 9?

DR. SHANK: Why not?

DR. MARKS: Yeah. Okay. Sure. Carcinogenicity, Page 12 in the discussion. The only other comment I had, here, the methyl sulfate, in Page 9 also; what is that about?

DR. HILL: I could tell you about that as soon as I get there.

DR. MARKS: I have Page 9. Where is it mentioned?

DR. HILL: I don't think it's on Page 9.

DR. MARKS: Impurities, compounds. In some of these test formulations -- sugar included -- Monomethyl sulfate was often a component in the test material.

DR. HILL: It's because they're using dimethyl sulfate, to methylate, to make that quaternary ammonium compound somewhere in the process toward the end.

DR. MARKS: So, that didn't raise -- there's no concerns the way that is?

DR. HILL: At those levels of use, it wouldn't be.

DR. MARKS: Okay. Good.

DR. HILL: And those are actually -- you're seeing it less all the time, but at least legacy drugs are still

some pharmaceuticals that have that monomethyl sulfate in there as the counterion. Like I said, less and less all the time. But that's still out there, so that didn't raise any concerns. But I flagged it.

DR. MARKS: Okay.

DR. HILL: There's something else. Hang on. It was not big, but --

MS. LORETZ: Back to the carcinogenicity just for a second. I mean, it reads -- it sounds bad. And that NICNAS report was not about assessing safe use of the hair dye, it had to do with labeling. And just -- it needs either context. Because I think it just leaves it with the impression of maybe there's a carcinogenicity concern. I think it's not what the NICNAS report --

DR. HILL: There might be. There might be.

DR. SHANK: And we can handle that in the discussion.

DR. HILL: So are you're saying take that language out since it doesn't pertain to Red 76 and put it in the discussion? Is that what you're suggesting? Which wouldn't --

MS. LORETZ: I think it needs --

DR. SHANK: No, it’s an impurity.

DR. HILL: I know. Well, it might or might not -- it can be produced by microbial reduction.

DR. SHANK: It’s in the dye at a very low concentration. We know the concentration. The International Agency for Research on Cancer has classified it as a possible human carcinogen. We need to discuss that. And this is a hair dye rinse-off. The compound is at a very low concentration to begin with.

DR. HILL: In the first place. Yep.

DR. SHANK: The systemic exposure would be extremely low; and it’s not a potent carcinogen like aflatoxin.

DR. HILL: You're talking about o-anisidine.

DR. SLAGA: Right.

DR. HILL: O-anisidine is a potential microbial metabolite or impurity.

DR. SHANK: Yes.

DR. HILL: I agree. The question is, do you even have a carcinogenicity section, that says anything more than no data on the compound were found? And then move all of that to the discussion.

DR. SHANK: Well, we have genotox.

DR. SLAGA: I don’t see any problem in leaving it there.

DR. HILL: Leaving it there?

DR. SLAGA: As long as we deal with the insufficient in the discussion.

DR. HILL: I didn’t raise it, it was raised down the table.

DR. SLAGA: Oh.

DR. MARKS: Oh, no, I raised it right in the beginning. I wanted to make sure we addressed it --

DR. SHANK: We have genotox.

DR. MARKS: -- in the discussion section. And, Linda, I think your further clarifying is good, and those points you make, Ron Shank, about it’s not a potent carcinogen, low concentrations. It’s a rinse-off. Anything else, Tom, you would -- SO, tomorrow, if it comes up, I’ll mention those points.

DR. SLAGA: Okay.

DR. HILL: But I still say, could you just move all of that to the discussion section? Because it’s not carcinogenicity data on that compound. There’s no data on

it. We have the genotox. And what's really here is discussion information, except they're all references. And if you don't like references in discussion, then you can't move it. I'm just leaving that hanging.

DR. MARKS: Well, Tom, are you okay with the way it is, and then just in the discussion address it?

DR. SLAGA: Yeah. I'm okay with it, as long as we discuss it.

DR. MARKS: Okay.

DR. SHANK: Just one comment in the genotox section. All of the exposure should be referred to as doses. In the Ames test, the dose was -- those aren't doses, those are concentrations.

DR. HILL: Okay. I flagged that too. Also, there's one that says 1000 milligram per mil, and that can't be right, it must be microgram, but check it.

DR. SHANK: Right.

DR. MARKS: Good. Any other comments? Let me close this. Triacetin, is that in the Admin one?

Dr. Belsito's Team

DR. BELSITO: If we're okay with that, at this point, then we need to move on to Basic Red, 76. So, this

is the first time we're looking at the safety assessment of the hair dye. I thought it was safe as used, but I just wanted the team's input into the very mixed genotoxicity data that existed for this. PDF 12 and Table 4.

DR. LIEBLER: I have flagged in the genotox section, in the middle of that paragraph, the test substance was not genotoxic, but was clastogenic at a dose of 1000 mg/mL -- that's a gram per mL -- without metabolic activation. I mean, that's so high as to be probably not a relevant test.

DR. BELSITO: Do we put that in the discussion?

DR. LIEBLER: I think it's reasonable. I'd like to hear what Tom Slaga's take is on some of these genotox results. I mean, the test methods are actually -- aside from the -- the AMES, there is a couple of positives in two strains. And then in one, two, three, four, five strains, up to very high dose, negative.

DR. EISENMANN: And what I read of the micronucleus assay, was positive at concentration where there was some precipitation out of the material.

DR. LIEBLER: And then in the in vivo test, negative. I think the body of data, overall, suggests that

this material is not genotoxic.

DR. BELSITO: I agree. I said safe as used, but that we discuss this.

DR. LIEBLER: Right. Yeah.

DR. BELSITO: Do we put this in the discussion as to why we're discounting the genotox data that's there?

DR. LIEBLER: Right. This reminds me a little bit of the methylxanthines where we had a mixed package of genotox data. And Tom stepped through it, very nicely, in our discussion last time; pointing out that there appeared to be a systematic difference. Having to do with when they used metabolism in the vitro test, and then all the in vivos were negative. And this is similar to what we saw with those.

DR. BELSITO: Okay. Priya, you have all that?

MS. CHERIAN: Yes. I have a question about the metabolites. Was there any concern about the aromatic amines.

DR. LIEBLER: Speak up into the microphone.

DR. BELSITO: Concern about metabolites and the aromatic amines.

DR. SNYDER: I think we have to expand on that a

little bit more under carcinogenicity in the discussion. And so, when there is an issue with those metabolites, the aromatic amines, the o-anisidine. I think we need to expand on it a little bit, why we're not concern. I think it's going to be in the context of exposure and how many even lacks to be present in the formulation. So, I think we can handle that. But I had a note that we need to, probably, have a little bit more in the discussion about that.

Because if you read it, it looks like we're indicating they're present and they can cause all these bad cancers, but it's in the context of dose and --

DR. EISENMANN: Frankly, I don't think they had any data. It's a general question using NICNAS' assessments when they're not -- I mean, this is coming up in a lot of CIR reports. Are they really necessary to put them in when they're not concerning cosmetic use? This is one of the reasons why you decided to do hair dyes, individually, rather than group, because they are metabolized very -- each one is very different.

And NICNAS is trying to put them all together, in saying they're all metabolized to these carcinogenic

compounds, just for hazard classification. For like shipping -- how they would label it if they were shipping a container of basic whatever, red 76. So, whether or not NICNAS' assessment actually need to be in the reports is, to me, a more general question.

DR. SNYDER: I think that's a good point.

DR. LIEBLER: So, the data that we have in our report, on ADME, is not specific with respect to metabolism per se. It's all basically absorption, and distribution, of radio label from administered compound. In those data, it does indicate that this material is very poorly absorbed. It's possible, in principle, for azo dyes to be reduced, I believe. Curt, correct me if I'm wrong, but I think that's mainly a gut bacteria biotransformation, azo dye reduction?

DR. KLAASSEN: Yeah, primarily, but the liver can do it as well.

DR. LIEBLER: Yeah. But there's no evidence that that's happening with this molecule. And the fact that it's very poorly absorbed suggests that the production of any possible aromatic amine metabolites, from this stuff, would be pretty low. I mean, it's really designed to have

competing metabolism, anyway, if you look at the structure. It's a terrific candidate for just elimination by conjugation of the aromatic hydroxyl group. But I don't know if there's any data that describes the metabolism of this compound at all.

DR. SNYDER: It is listed as an impurity though, under impurities.

DR. LIEBLER: Yes.

DR. SNYDER: And it's in Table 2.

DR. BELSITO: So, what do we do with that data? I mean, we put that in the discussion and then talk about the genotox data, and the very high levels at which any findings are seen? And then just dismiss it?

DR. LIEBLER: Yes.

DR. KLAASSEN: I think it was mentioned, the two people on the other team can answer about the mutagenicity, and help us with the wording there. I agree with what we're saying. But also the amine, Ron is the expert on that, and he can get us some confidence on the right wording, I think.

DR. BELSITO: Okay. So, we'll ask Dr. Slaga to provide the words for the discussion on that. Anything

else on this?

Combined Discussion

DR. MARKS: This is the first time we've seen this ingredient, Basic Red 76, which is a hair colorant and hair conditioning agent. Our team, after reviewing the data, felt that we could move for a tentative report with a safe conclusion.

DR. BERGFELD: And that's a motion?

DR. MARKS: Yes.

DR. BERGFELD: Is there a second?

DR. BELSITO: Second.

DR. BERGFELD: Any other comments?

DR. MARKS: Yeah, we felt in the pre-use open patch testing, on Page 9, that we could shorten that allergen alert test paragraph. We didn't need the whole methodology in that, condense it to a couple sentences. In the discussion, we wanted to address the carcinogenicity issue; that it's not a potent carcinogen that has low concentration, and it's used as a rinse-off. And that would all support the safety of it.

DR. BERGFELD: Any other comments for the discussion, or other editorial comments?

DR. BELSITO: Just could you be specific about the patch test application, Jim?

DR. MARKS: Yeah, Page 9. Let me see what I had. There was a pretty long paragraph, and I thought we could just mention that, indeed, this test existed. But we didn't have to put the whole paragraph; just include that this test really exist as a different method of patch testing that's been suggested as a pre-use.

DR. BELSITO: You're talking about the Coonrods' (phonetic) paper?

DR. MARKS: Yeah, the AAT. If you like that entire paragraph, that's the one that starts with "Furthermore, according to a report published in 2018" and that goes into some of the details. If you feel like you'd like to keep that whole paragraph, I'm not going to --

DR. BELSITO: So, that's the paragraph, the new one.

DR. MARKS: Yeah.

DR. BELSITO: Yeah, I mean, I think, that can certainly be shortened.

DR. MARKS: Yes,

DR. BELSITO: I thought you were talking about our recommendations for patch testing.

DR. MARKS: No.

DR. BELSITO: I think that will come up with -- because the question that I had is, do we change that based upon the European objection that application for 48 hours can result in sensitization. That's the whole point of Coonrods doing a 45-minute application, rinsing it off, and making it more real life. But, as I read our instructions, it says that sensitization 40 -- it said we recommend an open patch test be applied. We never said for how long. And that it be read at 48 hours. So, we're actually very vague in our recommendations. We don't say 45 minutes, we don't say it has to stay on for two days.

Alex pointed out that many of the manufacturers recommend that it stay on 48 hours; but our recommendation

does not specifically say how long the material should remain in the retroauricular area. So, I'm happy with the way we phrased it, particularly, in light of this recent paper and the European objections.

DR. MARKS: I agree.

DR. BERGFELD: Then the intent is to shorten this and make it a summary statement?

DR. BELSITO: To shorten the information coming from the Coonrods paper.

DR. MARKS: Correct. The one that's highlighted in yellow.

DR. BERGFELD: But it would be referenced, yes.

DR. MARKS: Yes.

DR. BELSITO: Yes, of course.

DR. BERGFELD: Yes. All right. Any other comments? Ron Hill?

DR. HILL: Yeah, human biochemistry, we don't always, with our human enzymes and such, reduce diazo compounds. Since the issue was raised that there might be

potential for metabolites to have carcinogenic character, and we don't have any information to suggest that the parent compound does. I don't know that there's anything known about whether microbes on the scalp can do that or not. Otherwise, we're raising something that might not be needing to be raised as an issue. And it's rinse-off anyway because it's hair dye use.

We might incidentally get a little bit of this caught in the upper layers of the scalp. We're suggesting something that might not even occur. But if there were information to suggest, yes, we know microbes on the skin and the scalp can do the reduction, or we don't see any evidence of that could occur that would be helpful to informing this. Where it is right now, we know in our gut we reduced this, but we're not swallowing it. To my knowledge, generally, humans don't reduce diazo compounds systemically. Sometimes they do, but it's compound specific.

DR. BERGFELD: Thank you. Alex, you want to comment on that or -- no.

MS. KOWCZ: I don't think we would have that

data.

DR. HILL: No. I'm sure not.

MS. BERGFELD: There's great interest in the microbiome of the skin right now; and so, it's a growing body of information.

DR. HILL: My point is that in humans we reduce those; in fact, that's classic prodrug stuff, but it always happens with microbes in the gut and not our own enzymes.

DR. BELSITO: Yeah. All right. I'm going to call the question then, since I see no other person looking like they want to comment. All those in favor? You want to comment?

DR. SHANK: I was voting.

DR. BERGFELD: You're voting. Okay. Get going. All those in favor of this conclusion of as safe. Thank you. Moving onto the next to the last ingredient in this group, the Benzyl Salicylate with Dr. Belsito.

Safety Assessment of Basic Red 76 as Used in Cosmetics

Status: Draft Final Report for Panel Review
Release Date: May 10, 2019
Panel Meeting Date: June 6-7, 2019

The 2019 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 Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Priya Cherian, Scientific Analyst/Writer.

1620 L Street, NW, Suite 1200 ◊ Washington, DC 20036-4702 ◊ ph 202.331.0651 ◊ fax 202.331.0088 ◊
cirinfo@cir-safety.org

ABSTRACT

The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) assessed the safety of Basic Red 76, which is reported to function in cosmetics as a hair colorant and hair-conditioning agent. The Panel reviewed the available data to determine the safety of this ingredient. The Panel concluded that Basic Red 76 is safe for use in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

This is a safety assessment of Basic Red 76 as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook (*wINCI; *Dictionary*), Basic Red 76 is a monoazo color that functions as a hair colorant and hair-conditioning agent.[1]

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

Much of the data included in this safety assessment was gathered from the opinions of European scientific committees, specifically, the Scientific Committee on Consumer Safety (SCCS)[2] and Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP).[3]

CHEMISTRY

Definition and Structure

As given in the *Dictionary*, Basic Red 76 (CAS No. 68391-30-0) is the azo dye that conforms to the following structure[1]:

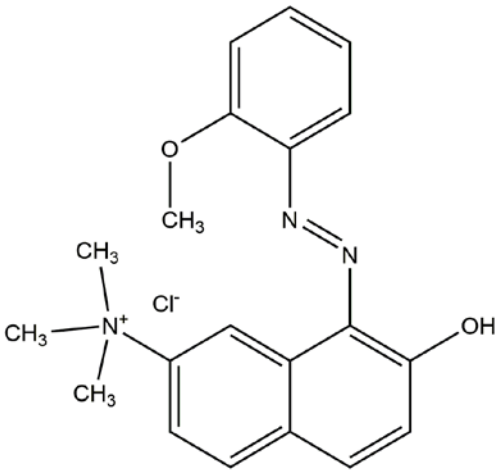


Figure 1. The azo dye, Basic Red 76

Physical and Chemical Properties

Basic Red 76 is a cationic direct dye that is water soluble.[2] Ultraviolet-visible (UV-Vis)-spectra showed maxima at 235 nm (62% Basic Red 76) and 332 nm (80% Basic Red 76). This ingredient, in its pure form, is a red powder with a melting point of 200°C and an octanol-water partitioning coefficient of -1.78. A list of chemical and physical properties for Basic Red 76 is provided in Table 1.

Method of Manufacture

While no methods were found in the publically available literature specific to the preparation of Basic Red 76, most azo dyes are synthesized in the same manner.[4] The first of two steps in the classic synthesis of dyes like Basic Red 76 involves the diazotization of a primary aromatic amine (e.g., 2-methoxyaniline), in a cold aqueous, acidic solution, with sodium nitrite. The resulting diazonium salt is highly reactive, and an arylazo-dehydrogenation reaction with an aromatic alcohol (e.g., 7-hydroxy-*N*,*N*,*N*-trimethylnaphthalen-2-aminium chloride) quickly results in an azo dye.

Impurities/Components

Based on data obtained from the SCCS, it appears that the materials tested were not always purely Basic Red 76, and had various chemical compositions.[2] In some of these test material formulations, sugars were included. Monomethyl sulfate was often a component in the test material that was used as an anion to the dye. Some other reported impurities/components of the test materials include *o*-anisidine, chloride, and sodium. Table 2 provides information on the components of the specific test materials used in the toxicity studies presented in this report. Throughout the report, please refer to Table 2 to note the full compositions of the test materials. In addition, Table 2 also provides composition information of Basic Red 76 as used in the market.

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics. Use frequencies of individual ingredients in cosmetics are collected from manufacturers and reported by cosmetic product category in the FDA Voluntary Cosmetic Registration Program (VCRP) database. Use concentration data are submitted by the cosmetic industry in response to a survey, conducted by the Personal Care Products Council (Council), of maximum reported use concentrations by product category.

According to 2019 VCRP survey data, Basic Red 76 is reported to be used in 48 hair-coloring formulations and 2 nail polish and enamel formulations.[5] The results of the concentration of use survey conducted by the Council indicate that the highest concentration of use reported for Basic Red 76 was 0.35% in hair dyes and colors.[6] Concentration of use data were not provided for use in nail products. Detailed data regarding concentration and frequency of use can be reviewed in Table 3.

Basic Red 76 is considered a coal tar hair dye for which regulations require caution statements and instructions regarding patch tests in order to be exempt from certain adulteration and color additive provisions of the US Federal Food, Drug, and Cosmetic Act. In order to be exempt, the following caution statement must be displayed on all coal tar hair dye products:

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

Product labels shall also bear 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.

In 2012, a report was published regarding such self-testing for contact sensitization to hair dyes.[7] These authors concluded that, in its present form, the hair dye self-test has severe limitations. An accompanying editorial performed on behalf of the European Society of Contact Dermatitis (ESCD) 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, stating 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.[8]

Additionally, according to a report published in 2018, a different method of patch-testing was suggested utilizing more relevant protocols regarding exposure time and test preparation that reflect the actual use conditions with hair dying.[9] According to this study, a self-test protocol for an allergy alert test (AAT) was developed that elicits a self-noticeable alert signal in *p*-phenylenediamine (PPD)-allergic consumers after a 45 minute exposure to the hair dye mixed with developer.

Basic Red 76 is listed in the EU Cosmetics Regulation 1197/2013 Annex III, and is allowed in non-oxidative hair dye products at a maximum concentration of 2%.[10] According to the SCCS, Basic Red 76 containing up to18% methyl sulfate does not pose a risk to the health of the consumer when used as a non-oxidative hair dye with a maximum head-on concentration of 2.0%.[2]

TOXICOKINETIC STUDIES

Azo bond cleavage and reduction is mediated by enzymes found in the liver, skin, and intestines.[11] Responsible cofactors and enzymes include nicotinamide adenine dinucleotide (NADH), cytochrome P450 reductase, and NAD(P)H quinone oxidoreductase. Both skin and intestinal microflora have been shown to reduce azo linkage, forming aromatic amines (e.g., *o*-anisidine). The produced aromatic amines can potentially have a greater expected absorption rate than the dye from which they are derived from.

Dermal Penetration

Animal

A two-part dermal/percutaneous absorption study was performed according to Organization for Economic Cooperation and Development (OECD) test guideline (TG) 428.[2,12] Four replicates from each animal (one male, one female) of dermatomed pig skin, 0.75 mm thick, were used per experiment. In experiment A, 2% test material (80.5% Basic Red 76) in direct dye was applied to skin samples. Experiment B involved 2% test material (80.5% Basic Red 76) in water. In both experiments, applications of approximately 20 mg/cm^2 were applied to the skin. Skin discs of 1.0 cm^2 were exposed to the test substance for 30 minutes, and then rinsed. The receptor fluid used was a phosphate buffered saline. Basic Red 76, at concentrations of approximately 1.44%, 0.046%, and 0.0049% was present in the stratum corneum, epidermis/dermis, and receptor fluid, respectively. Samples treated with the aqueous solution displayed penetration amounts of 3.87% in the stratum corneum, 1.77% in the epidermis/dermis, and 0.012% in the receptor fluid. The amount of the test substance that was considered bioavailable from the direct dye cream and the aqueous solution was 1.96 ± 0.83 µg/cm^2 and 6.52 ± 3.58 µg/cm^2, respectively.

Human

Ten male subjects had 20 µL of a 1 mM test material (55.5% Basic Red 76), in 40% aqueous isopropanol, applied to five separate areas (5.3 cm^2) of the inner forearm.[3] The dye stains were removed by ten repeated strippings with tape after 10 minutes, 24, 48, and 72 hours, and the amount of dye that potentially penetrated was estimated. The dye was not suspected to have been diffused into the horny layer, and the researchers concluded that the dye was not absorbed by the skin. No other information regarding this study was provided.

Absorption, Distribution, Metabolism, and Excretion (ADME)

Animal

Dermal

An application of 200 µL of a hair setting lotion containing 0.1% test material (55.5% [^{14}C]-labelled Basic Red 76, labeling regiochemistry not stated) was applied to the skin of 3 Sprague-Dawley rats over an area of 1.5” x 1.5”, corresponding to an exposure of 31.3 mg/cm^2 skin (and 31.3 µg Basic Red 76/cm^2 skin).[3] The rats were anesthetized for 1 hour after application, then they were fitted with a collar to prevent licking. Radioactivity recovered from feces and urine was less than 0.2% and 0.3% of the applied dose, respectively. A maximum total absorption was calculated to be 0.5%, corresponding to a maximum of 0.15 µg/cm^2 of skin. Excretion of radioactivity in urine and feces was measured for 24 hours after application. The amount of radioactivity recovered in the carcass or organs was not determined.

Other studies were performed using a setting lotion and shampoo formulation containing 0.2 and 0.5% test material (55.5% [^{14}C]-labelled Basic Red 76, labeling regiochemistry not stated), respectively.[3] Application was performed on the clipped (but not shaven) skin of Wistar rats. After an application of 100 µL to 5 Wistar rats/sex, treatment sites were covered with a non-occlusive glass capsule containing small holes. Exposure occurred for 24 hours. In rats dosed with the setting lotion formulation containing 0.2% of the test material (25 µg of test material/cm^2 skin), more than 80% of the applied radioactivity was recovered on the hair, and about 10% was recovered on the skin. The radioactivity recovered in the urine and feces was 0.07 and 0.16%, respectively. No radioactivity was detected in the carcasses. In a different study where rats were exposed for 24 hours to 70 and 140 µL of a shampoo containing 0.5% test material (55.5% [^{14}C]-labelled Basic Red 76, labeling regiochemistry not stated), $\geq$ 93% of the applied radioactivity was recovered in the hair rinsings. Approximately 2.1 and 1.7% of the radioactivity was recovered on the treated skin in males and females, respectively. The radioactivity recovered in the urine was less than 0.007% in males and 0.002% in females. Less than 10% of the applied radioactivity was observed in the feces of treated animals. The amount of radioactivity recovered in the carcass or organs was not determined.

Parenteral

Three male Wistar rats were given a single intravenous (i.v.) dose of 2.5 mg/kg bw of the test material (55.5% [^{14}C]-labelled Basic Red 76, labeling regiochemistry not stated) in physiological saline.[3] Approximately 63 and 15% of the administered test substance was recovered in the feces and urine, respectively, over a duration of 24 hours. The level of radioactivity detected in the carcass 24 hours after the administrated dose was approximately 9%. In another study, mice were given a single subcutaneous dose of 5 mg/kg of the same test substance. Two minutes after administration, 31% of the radioactivity was present in the liver and kidneys, 9% in the small intestine, and 1.3% in the lungs. After 24 hours, the total radioactivity in the liver, kidneys and lungs decreased to 33.7% of the given dose. Specific radioactivity was highest in the cecum and large intestine by the end of the study.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Oral

In an acute toxicity study, 3 CF1 mice were treated with a single oral dose of 1, 2.51, or 5.01 g/kg bw test material (55.5% Basic Red 76) in a volume of 20 to 40 mL/kg, and 10 male mice received the top dose of 10 mg/kg bw (method of oral dosing was not provided). [3] The animals were observed for 7 days after treatment. Lethargy and breathing disorders were observed in mice given 10 g/kg bw of the test substance. The LD_{50} value was reported to be > 10 g/kg bw.

Wistar rats (3/sex) were given a single oral dose of 2 g/kg bw test material (62% Basic Red 76) in propylene glycol via gavage.[2,13] Three male and one female rat displayed hunched posture on the first day of treatment. Red staining of the back and/or snout and/or head was observed in one female and two male mice. Red and/or yellow feces and/or urine were seen in all animals. The established oral LD_{50} value was > 2 g/kg bw.

CFY rats (2/sex/group) were given a single oral dose (0, 0.1, 1, 4, 8, or 16 g/kg bw) of the test material (55.5% Basic Red 76) in 1% aqueous methylcellulose (method of oral dosing was not provided).[3] Animals were observed for 14 days after treatment. All animals survived treatment. Lethargy, piloerection, decreased respiratory rate, and hunched posture were observed. At the 8 and 16 g/kg bw dose levels, red staining of the urine and feces was noted. The acute lethal dose of the test substance was reported to be > 16 g/kg bw.

Short-Term Toxicity Studies

Oral

Wistar MuRa Han 67 SPF rats (20/sex/group) were given 0 or 200 mg/kg bw of the test material (55.5% Basic Red 76) in a volume of 10 mL/kg water via gavage 5 days per week for 12 weeks in a screening study.[3] All animals survived the duration of the experiment. Aggressive behavior was apparent in all dosed animals. In males, body weight gain was similar to the control group, however, in females, slight but significantly lower mean body weights were recorded (95 - 96% of control) on the 5th, 7th, 9th, and 12th week, and at the end of the study. Colored urine was observed in all dosed animals. Increases in the mean cell volume and hematocrit values were noted in male rats and some female rats. In male rats, a slight increase in cerebral weights, as compared to the control groups, was observed. In females, kidney, heart, and liver weights were lower than those of control animals. The no-observable-adverse-effect-level (NOAEL) was reported to be < 200 mg/kg bw/d.

Subchronic Toxicity Studies

Oral

Groups of 10 female and 10 male Sprague-Dawley CD rats were dosed with 0 and 20 mg/kg bw of the test material (55.5% Basic Red 76), in a volume of 10 mL/kg aqueous solution.[3] The test article was administered 5 days/week for 13 weeks by gavage. No mortalities were reported. Body weight gain was similar in the control and treated groups. No other effects were noted. The dose of 20 mg/kg bw/d was determined to be a “no effect level.”

SPF-bred Wistar rats (12/sex/group) were given a single daily dose of the test material (80.5% Basic Red 76) in distilled water via gavage for 90 days.[2,14] Rats received doses of 0, 60, 250, or 1000 mg/kg bw/d. One female dosed with 60 mg/kg bw/d was found dead on day 60, however a gavage error was considered to be the cause of death. Staining of body parts and discoloration of the feces/urine was observed. Infrequent and intermittent clonic spasms were observed in some test animals in all dose groups. No relevant body weight or food intake level changes were noted. Destruction of red blood cells, increased tissue iron in the spleen and liver, and increased serum bilirubin levels were noted in animals dosed with 250 and 1000 mg/kg bw/d. Thyroid follicular cell hypertrophy and adenohypophyseal cell hypertrophy was observed in rats given 1000 mg/kg bw/d; however, this effect is not considered relevant to humans as rats have a significantly higher sensitivity to this effect. At 60 mg/kg bw/day, decreased red blood count cells, hemoglobin levels, hematocrit levels, and mean corpuscular hemoglobin concentrations were seen. The NOAEL was reported to be 60 mg/kg/d. This study also included recovery groups of 5 rats/sex/dose group. The animals in these groups were examined after treatment, for four weeks. The hematological effects observed in the treated animals were widely resolved during the 28-day recovery period.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Doses of 0, 60, 250, and 1000 mg/kg bw/d of the test substance (89.1% Basic Red 76) were given via gavage to Wistar rats (24/group) on days 6 to 20 of gestation.[2,15] All females were killed and examined 21 days after mating. In rats treated with 60 mg/kg bw/d, no signs of developmental toxicity were observed. At the 250 mg/kg bw/d dose level, decreased body weight, weight gain, and food consumption was observed in maternal rats; a decrease in fetal body weight was also reported. Similar findings were seen in rats treated with 1000 mg/kg bw/d, however, signs of toxicity were more pronounced in the

group treated with 250 mg/kg bw/d. Slight increases in the thinning of the central tendon region of the diaphragm and left-sided umbilical artery was observed in offspring. The maternal and developmental NOAEL was determined to be 60 mg/kg bw/d. Another study was performed using Sprague-Dawley CD rats.[3] Rats were given the test substance (55.5% Basic Red 76) in distilled water in doses of either 0 (20 rats) or 50 mg/kg (25 rats) bw/d on days 6 - 15 of gestation via gavage. On day 20 of gestation, the dams were killed. No adverse effects were reported in dams or fetuses treated with 50 mg/kg bw/d.

GENOTOXICITY

Details of the genotoxicity studies summarized below are provided in Table 4. (Information regarding the test material composition in these studies is provided in Table 2.)

Basic Red 76 was generally not genotoxic. Negative results were observed in Ames tests at concentrations of up to 5000 µg/plate, with or without metabolic activation (*Salmonella typhimurium* TA98, TA100, TA102, TA1535, and TA1537**)**.[2,3] However, in another study, positive results were observed in an Ames test using *S. typhimurium* in strains TA1537 and TA1538 (test concentration not specified), while negative results were observed in strains TA98, TA100, and TA1535 in concentrations as high as 5000 µg/plate.[3] Negative results were obtained in mammalian gene mutation assays using Chinese hamster V79 cells (dose not specified) and mouse lymphoma L5178Y cells at doses up to 318 µg/mL, with and without metabolic activation, and 425 µg/mL, with and without metabolic activation.[3,16] The test substance was not genotoxic in a mammalian chromosome aberration assay using Chinese hamster V79 cells at concentrations of up to 500 mg/mL, but it was clastogenic at a dose of 1000 mg/mL without metabolic activation.[3] The test substance was clastogenic in an in vitro micronucleus test in V79 cells at concentrations as low as 212.5 µg/mL with and without metabolic activation.[16] Positive results were observed mainly at concentrations associated with test material precipitation. In in vivo micronucleus assays in mice, the test substance was not clastogenic at concentrations of up to 5000 mg/kg given orally to mice.[3,17]

CARCINOGENICITY

Information regarding the carcinogenicity of Basic Red 76 was not found, however the potential metabolites of Basic Red 76, such as *o*-anisidine, have induced a number of multi-organ tumors according to third party summaries of animal studies cited by National Industrial Chemicals Notification and Assessment Scheme (NICNAS).[11] Both malignant and benign tumors in the bladder, spleen, subcutaneous tissues, kidneys, adrenal gland, liver, mammary glands, skin, blood, blood vessels, thyroid, lungs, gallbladder and renal pelvis, have been associated with the exposure of the metabolized aromatic amines. NICNAS, however, did not provide any specific evidence stating that Basic Red 76 is metabolized to *o*-anisidine.

DERMAL IRRITATION AND SENSITIZATION

Irritation

Animal

A skin irritation test was performed according to OECD TG 404.[2,18] A semi-occlusive patch containing 500 mg of the test substance (62% Basic Red 76) was applied to approximately 150 cm^2 of shaved skin of 3 New Zealand White rabbits. Patches were removed after 4 hours. Skin was evaluated 1, 24, 48 and 72 hours, as well as 7, 10 and 14 days after removal of dressing. No visible signs of irritation were observed. Minimal red staining was noted in all animals.

In another study, a 24-h occlusive patch of 0.5 g undiluted test material (62% Basic Red 76) was applied to a 1 in^2 of intact or scarified skin of the back of 3 New Zealand White rabbits.[3] No reactions were reported. A similar study was performed with the same test substance that was dampened for adhesion. A dose of 0.5 g of the test material (62% Basic Red 76) was dampened with 0.5 mL distilled water and applied to a 1 in^2 area of intact or scarified skin of the back of 3 New Zealand White rabbits. No reactions were recorded.

Sensitization

Animal

Twenty-five µL of the test material (62% Basic Red 76) was applied in concentrations of 2.5, 5, and 10% in a 7:3 v/v ethanol:water mixture.[2,19] Ten percent was the highest technically applicable concentration in the vehicle. Applications were made on the earlobes of mice (4 females/ group) in a local lymph node assay (LLNA). The test substance was applied once daily for 3 days. Five days after the first treatment, mice were given an intravenous injection of radiolabelled thymidine. Mice were killed 5 hours after thymidine administration. The draining lymph nodes were excised, pooled, placed in scintillation vials, and tested for proliferative capacity. The stimulation index values were 0.9, 1.1, and 1.3, for the 2.5, 5, and 10% dose levels, respectively. The test material was considered to be non-sensitizing.

In a Magnusson-Kligman test performed according to OECD TG 406, the sensitization potential of the test material (55.5% Basic Red 76) was evaluated using 10 female Dunkin-Hartley guinea pigs.[3] Intradermal induction consisted of injections of the material solution (0.1% test material in water), Freund's Complete Adjuvant (FCA) diluted with an equal volume of water, and a 1:1 mixture of the material solution and FCA. One week after the administration of injections, a solution of 75% w/v of the test substance in distilled water was applied to the skin. A challenge patch was applied 2 weeks later at a concentration of 25% w/v of the test material. Irritation was noted after administration of the intradermal injection in all animals, which was still present at the time of topical induction. Half of the test animals displayed erythema after the challenge phase that was resolved by 48 hours. The sensitization potential was considered to be equivocal.

OCULAR IRRITATION STUDIES

Animal

An ocular irritation test was performed according to OECD TG 405.[2,20] The test material (62% Basic Red 76; 0.1 g) was instilled into the conjunctival sac of 3 New Zealand White rabbits. Treated eyes were rinsed following a 24 hour exposure period. Scoring occurred 1, 24, 48, and 72 hours, and 7 days, after instillation. Redness of the conjunctivae and sclerae, discharge, and chemosis were apparent at the beginning of treatment, but were no longer present after 72 hours. Minimal staining of the eyes was observed after 1 hour and 24 hours in all subjects. Staining was present in two animals at the 48 hour mark, and in one animal at the 72 hour mark. No abnormalities or corrosion was reported in the cornea or iris of test animals.

In a similar study using 3 New Zealand White rabbits, the test material (55.5% Basic Red 76) in physiological saline was instilled into the conjunctival sac (0.1 mL) of one eye of each rabbit.[3] The concentration of the test substance used was 0.5%. Eye reactions were recorded after 30 and 60 minutes, and 24 and 48 hours. No effects on the cornea or iris were reported, however, discoloration was noted.

HAIR DYE EPIDEMIOLOGY

Hair dyes may be broadly grouped into oxidative (permanent) and direct (semipermanent) hair dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes are a preformed color. Basic Red 76 is a direct hair dye ingredient. While the safety of individual hair dye ingredients is not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. The CIR Expert Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer. A detailed summary of the available hair dye epidemiology data is available at https://www.cir-safety.org/cir-findings.

SUMMARY

Basic Red 76 is a monoazo color that is reported to function as a hair colorant and hair-conditioning agent. Synthesis of the dye includes diazotization of a primary aromatic amine in a cold, aqueous, acidic solution with sodium nitrite. The resulting diazonium salt is highly reactive and an arylazo-dehydrogenation reaction with an aromatic alcohol quickly results in an azo dye.

According to 2019 VCRP survey data, Basic Red 76 is reported to be used in 48 hair-coloring formulations and 2 nail polish and enamel formulations. The results of the concentration of use survey conducted by the Council indicate that the highest concentration of use reported for Basic Red 76 was 0.35% in hair dyes and colors. Basic Red 76 is listed in the EU Cosmetics Regulation 1223/2009 Annex III, and is allowed in non-oxidative hair dye products at a maximum concentration of 2.0%.

In a dermal absorption study involving pig skin, 2% test material (80.5% Basic Red 76) was applied to samples in either a direct dye cream or aqueous formulation. Applications were performed in amounts of approximately 20 mg/cm^2. The amount of the test material that was considered to be bioavailable by the direct dye cream and the aqueous solution was 1.96 ± 0.83 $\mu g/cm^2$ and 6.52 ± 3.58 $\mu g/cm^2$, respectively. In a human study, 20 µL of 1 mM of the test substance (55.5% Basic Red 76) in aqueous isopropanol was applied to the forearm. The dye stains were removed by ten repeated strippings with tape after 10 minutes, 24, 48, and 72 hours. The dye was not suspected to have diffused into the horny layer.

When an application of 200 µL of a hair setting lotion containing 0.1% test material (55.5% [^{14}C]-labelled Basic Red 76) was applied to the skin of rats over an area of 1.5" x 1.5," the amount of radioactivity recovered from feces and urine (measured for 24 hours after application) was less than 0.2% and 0.3% of the applied dose, respectively. A maximum total absorption was calculated to be 0.5%, corresponding to a maximum of 0.15 $\mu g/cm^2$ of skin. Other studies were performed in which a setting lotion and shampoo formulation containing 0.2 and 0.5% test material (55.5% [^{14}C]-labelled Basic Red 76), respectively, were applied to the skin of Wistar rats for 24 hours.

In rats dosed with 0.2% test material (55.5% [^{14}C]-labelled Basic Red 76), over 80% of the applied radioactivity was recovered on the hair. The radioactivity recovered in the urine and feces was 0.07 and 0.16%, respectively. In rats treated with the formulation containing 0.5% test material, 93-102% of the applied radioactivity was recovered in hair rinsings. Approximately 2.1 and 1.7% radioactivity was recovered on the skin treated with 70 and 140 µL of the shampoo, respectively. The radioactivity recovered in the urine was < 0.007% in males and 0.002% in females. Less than 10% of the applied radioactivity was observed in the feces of treated animals. Three male Wistar rats were given a single i.v. dose of 2.5 mg/kg bw of test material (55.5% [^{14}C]-labelled Basic Red 76) in physiological saline. Approximately 63 and 15% of the administered dose was recovered in the feces and urine, respectively. In a similar study, mice were given a single subcutaneous dose of 5 mg/kg of the same test substance. Two minutes after administration, 31% radioactivity was present in the liver and kidneys, 9% in the small intestine, and 1.4% in the lungs. After 24 hours, the total radioactivity in the liver, kidneys and lungs decreased to 33.7% of the given dose.

The oral LD_{50} of a test substance containing Basic Red 76 was > 10 g/kg bw (55.5% Basic Red 76) in CF1 mice, > 2 g/kg bw (62% Basic Red 76) in Wistar rats and > 16 g/kg (55.5% Basic Red 76) bw in CFY rats. These values were the highest doses tested in each study. Some signs of toxicity were observed.

Decreases in organ weights and increases in the mean cell volume and hematocrit values were noted when Wistar MuRa Han 67 SPF rats were dosed via gavage 5 days a week for 12 weeks (test material, 55.5% Basic Red 76; 200 mg/kg bw). The dose of 20 mg/kg bw/d was determined to be a no effect level in Sprague-Dawley CD rats dosed by gavage for 5 days/week for 13 weeks (test material, 55.5% Basic Red 76). Toxic effects included lowered body and organ weights. The established NOAEL in a 90-day study involving Wistar rats dosed via gavage was 60 mg/kg/d (test material, 80.5% Basic Red 76).

No signs of developmental toxicity were observed in Wistar rats given 60 mg/kg bw/d on days 6 – 20 of gestation via gavage (test material, 89.1% Basic Red 76); however, toxic effects were noted at the 250 mg/kg bw/d dose level and higher when the same test substance was used. The maternal and developmental NOAEL was determined to be 60 mg/kg bw/d. In a different study, no adverse effects were reported in Sprague-Dawley CD rats given up to 50 mg/kg bw/d via gavage on gestation days 6 - 15 (test substance, 55.5% Basic Red 76).

Mixed results were seen in Ames tests and mammalian gene mutation assays using Chinese hamster V79 cells with and without metabolic activation. Negative results were seen in a mammalian cell gene mutation assay using mouse lymphoma L5178Y cells. A chromosomal aberration assay using Chinese hamster V79 cells yielded negative results at up to 500 mg/mL; however, at the 1000 mg/mL concentration level, without metabolic activation, Basic Red 76 was clastogenic. An in vitro micronucleus test in V79 cells yielded positive results at concentrations as low as 212.5 µg/mL, with and without metabolic activation; however, in part one of the same experiment, negative results were seen at concentrations as high as 300 µg/mL with metabolic activation. Negative results were observed in in vivo micronucleus assays in mice at up to 5000 mg/kg.

No information regarding the carcinogenicity of Basic Red 76 was found. However, possible metabolites of Basic Red 76 (e.g., *o*-anisidine) have induced a number of multi-organ tumors according to several animal studies cited by NICNAS. (NICNAS, however, did not provide any specific evidence stating that Basic Red 76 is metabolized to *o*-anisidine.)

No irritation was reported when New Zealand White rabbits were dermally dosed with 500 mg or 0.5 g/in^2 of the test substance (62% Basic Red 76) under an occlusive patch. In an LLNA, Basic Red 76 was considered a non-sensitizer when 25 µL of the test substance (62% Basic Red 76) was applied to mouse earlobes at a concentration of up to 10%. In a Magnusson-Kligman test, the sensitization potential of the test material (55.5% Basic Red 76) was evaluated using Dunkin-Hartley guinea pigs. Intradermal induction consisted of injections of the material solution (0.1% test material in water), Freund's Complete Adjuvant (FCA) diluted with an equal volume of water, and a 1:1 mixture of the material solution and FCA. One week after the administration of injections, a solution of 75% w/v of the test substance in distilled water was applied to the skin. A challenge patch was applied 2 weeks later at a concentration of 25% w/v of the test material. Half of the test animals displayed erythema after the challenge phase that was resolved by 48 hours.

Ocular irritation was observed in New Zealand White rabbits following instillation of 0.1 g of the test material (62% Basic Red 76) into the conjunctival sac; this effect was resolved within 72 hours. In a different study, no irritation was reported when the test substance (55.5% Basic Red 76), at a concentration of 0.5%, in physiological saline, was placed in the conjunctival sac of New Zealand White rabbits.

The most recent comprehensive 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 summary of the available hair dye epidemiology data is available in the appropriate CIR Resource Document at https://www.cir-safety.org/cir-findings.

DISCUSSION

Basic Red 76 is a direct dye that is reported to function as a hair-colorant and hair-conditioning agent. The Panel found that the systemic toxicity, developmental/reproductive toxicity, genotoxicity, and irritation data in this report were sufficient. The Panel recognized the positive results of some genotoxicity studies, but considered them to be potentially misleading. Positive results were only obtained in in vitro studies, and at concentrations much higher than what would be used in cosmetics. All in vivo genotoxicity assays performed using Basic Red 76 yielded negative results, suggesting that the positive results seen in in vitro studies were not of concern. In addition, the Panel noted the carcinogenic potential of the aromatic amines formed by the metabolism of Basic Red 76. The concern regarding these metabolites was mitigated considering Basic Red 76 is poorly absorbed, used in rinse-off products, and is used at very low concentrations. In addition, because the ingredient is minimally absorbed, the actual exposure to these metabolized aromatic amines would be insignificant in light of cosmetic use conditions.

The Panel also considered the current recommendations for patch-testing, and noted that concerns over patch testing are on-going. New methods for patch-testing using relevant protocols regarding application time and actual use conditions are continuously being developed. The Panel suggests that the patch-test instructions provided by manufacturers should be followed before hair dye use. In addition, hair dyes containing Basic Red 76, 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 and other endpoints. Further details regarding hair dye epidemiology may found in the CIR Resource Document.

CONCLUSION

The CIR Expert Panel concluded that Basic Red 76 is safe in cosmetics in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Physical and Chemical Properties[2]

Property	Value
Physical Form	fine powder
Color	red
Molecular Weight (g/mol)	371.86
Melting Point (ºC)	200
Water Solubility (g/L @ room temperature)	10 – 100
Ethanol Solubility (g/L @ room temperature)	0.3 - 3
DMSO Solubility (g/L @ room temperature)	1 - 10
log P_{ow}	-1.7834 ± 0.1131
Ultraviolet absorption λ maxima (nm)	235, 332, 503

Table 2. Impurities/Components

Basic Red 76 (% w/w)	62[2]	80.5[2]	89.1[2]	55.5 (as chloride)[3]	> 77 (material used in the market)[2]
Water (% w/w)	5.1	4.1	3.1	NR	< 6
Monomethyl sulphate (% w/w)	11.8	15.9	11.4	NR	< 18
o-anisidine (%)	0.0005	0.0019	0.0011	NR	< 0.001
Chloromethane (% w/w)	0	0.3	0.1	NR	NR
Methyl acetate (% w/w)	0	0.1	NR	NR	NR
Methyl formate (% w/w)	0	0.4	NR	NR	NR
7-Hydroxy-*N*,*N*,*N*-trimethylnaphthalen-2-aminium chloride (%)	0	< 0.05	< 0.05	NR	NR
Methanol (% w/w)	0	0	0.7	NR	NR
Sulphated ash (% w/w)	0.4	0.3	0.1	NR	<5
Chloride (% w/w)	1.6	2.7	4.4	NR	<5
Sodium (%)	0.063	0.019	0.024	NR	NR
Calcium (%)	0.059	0	NR	NR	NR
Saccharose (% w/w)	25.8	0	NR	NR	NR
Sugar (undefined (%))	NR	NR	NR	16	NR
Volatile matter/water of crystallization (undefined (%))	NR	NR	NR	14	NR
Inorganic salts – chloride, sulfate, etc. (undefined (%))	NR	NR	NR	up to 100%	NR

NR = Not Reported

Table 3. Frequency (2019) and Concentration of Use (2017) of Basic Red 76

	# of Uses[5]	Conc of Use (%)[6]
Totals*	**48**	**0.057 - 0.35**
FDA Product Categories		
Hair Dyes and Colors	5	0.057 – 0.35
Hair Tints	3	0.18
Hair Shampoos (coloring)	11	0.2
Other Hair Coloring Preparations	5	0.13
Hair Rinses (Coloring)	24	NR
Nail Polish and Enamel	2	NR

NR = no reported use

Table 4. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System/Organism	Procedure	Results	Reference
IN VITRO						
80.5% Basic Red 76	Experiment 1: 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate Experiment 2: 33, 100, 333, 1000, 2500 and 5000 µg/plate	DMSO	*Salmonella typhimurium* (TA98, TA100, TA102, TA1535, and TA1537)	Bacterial reverse mutation assay; Experiment 1 involved direct plate incorporation with a 48 hour incubation time with and without S9 mix. Experiment 2 involved the same dosing, excluding the 10 µg/plate level. Cells were pre-incubated for 60 minutes and at least 48 hours of incubation with and without S9 mix.	Non-mutagenic	[2,21]
55.5% Basic Red 76	NR	NR	*S. typhimurium* (TA98, TA100, TA1535, TA1537, TA1538)	Bacterial reverse mutation assay; with and without metabolic activation.	The test substance induced mutation in TA1537 with metabolic activation and in TA1538 with and without metabolic activation.	[3]
80.5% Basic Red 76	Experiment 1: 26.6, 53.1, 106.3, 212.5, 318.8 µg/ml Experiment 2: 53.1, 106.3, 212.5, 318.8, 425 µg/ml	Deionized water	Mouse lymphoma cell line L5178Y	Mammalian cell gene mutation assay; Experiment 1: with and without S9-mix; treated for 4 hours; expression period of 72 hours Experiment 2: without S9-mix; treated for 24 hours; expression period of 48 hours A pre-test treatment of up to 1700 µg/ml with and without metabolic activation was also performed.	Non-mutagenic	[2,22]
55.5% Basic Red 76	NR	NR	Chinese hamster V79 cells	Mammalian cell gene mutation assay; V79 cells were treated with Basic Red 76 with and without metabolic activation	The test substance did not induce gene mutation in the V79 cells.	[3]
55.5% Basic Red 76	Experiment 1 (part 1): 62.5, 125, 250 mg/mL Experiment 1 (part 2): 250, 500, 1000 mg/mL Experiment 1 (part 3): 50, 100, 200 mg/mL Experiment 2 (part 1): 62.5, 125, 250 mg/mL Experiment 2 (part 2): 31.3, 62.5, 250 mg/mL	NR	Chinese hamster lung V79 cells	Mammalian chromosome aberration test; Experiment 1 (part 1): cells treated in absence of S9 for 18 h Experiment 1 (part 2): cells treated in absence of S9 for 28 h Experiment 2(part 3): cells treated in absence of S9 for 18 h Experiment 2 (part 1 and 2): cells treated in presence of S9 (duration not stated)	Non clastogenic at the 62.5, 125, 250, and 500 mg/mL levels; Clastogenic at the 1000 mg/mL level; a slight but significant increase in the frequency of chromosomal aberrations was present.	[3]
80.5% Basic Red 76	Experiment 1 (part 1): 53.1, 106.3, 212.5 µg/ml Experiment 1 (part 2): 150, 200, 300 µg/ml Experiment 2 (part 1): 106.3, 212.5, 425, 850, 1700 µg/ml Experiment 2 (part 2): 106.3, 212.5, 425 µg/ml	Deionized water	Chinese hamster V79 Cells	Micronucleus test; Experiment 1 (part 1): with and without S9-mix, treated for 4 hours; harvest time 24 hours after beginning of treatment Experiment 1 (part 2): with S9-mix, treated for 4 hours; harvest time 24 hours after beginning of treatment Experiment 2 (part 1): without S9-mix; treated for 20 hours, harvest time 24 hours after beginning of treatment Experiment 2 (part 2): with S-9 mix; treated for 4 hours, harvest time 48 hours after the beginning of treatment	Clastogenic In experiment 1, without metabolic activation, increases in cells with micronuclei were not noted. In experiment 1 (part 1), without metabolic activation, a biologically relevant increase in cells with micronuclei was not observed Dose-dependent, biologically relevant increases in micronuclei were observed in experiment 2 with and without metabolic activation. Micronuclei induction was observed at concentrations associated with test item precipitation (212.5, 425, 850 µg/ml)	[2,16]

Table 4. Genotoxicity studies

Test Article	Concentration/Dose	Vehicle	Test System/Organism	Procedure	Results	Reference
IN VIVO						
80.5% Basic Red 76	0, 25, 50, 100 mg/kg bw (oral); 200 mg/kg bw (intraperitoneal)	Deionized water	NMRI mice (5 mice/sex/group)	Micronucleus test; Mice were given oral doses or intraperitoneal doses of Basic Red 76. Bone marrow cells were collected 24 and 48 hours after a single administration. Toxicity was determined by measuring the ration of PCE and TE.	Non- clastogenic	[2,17]
55.5% Basic Red 76	5000 mg/kg bw	NR	CFW 1 mice (5/sex/group)	Micronucleus test; Mice were given doses of Basic Red 76 via gavage. This study was performed according to OECD 474. Duration of dosing was not provided. Animals were sacrificed at 24, 48, and 72 hours after treatment.	Non-clastogenic	[3]

NR = Not Reported; DMSO = dimethyl sulfoxide, PCE = polychromatic erythrocyte; TE = total erythrocytes

REFERENCES

1. Nikitakis J and Lange B (eds). Web-Based Ingredient Dictionary (wINCI). http://webdictionary.personalcarecouncil.org/jsp/IngredientSearchPage.jsp. Washington, D.C. Last Updated 2018. Date Accessed 9-6-2018.

2. Scientific Committee on Consumer Safety (SCCS). Opinion on Basic Red 76. 3-22-2011. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_051.pdf. Date Accessed 5-22-2018.

3. Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (SCCNFP). Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. 3-18-2003. http://ec.europa.eu/health/archive/ph_risk/committees/sccp/documents/out_197.pdf. Date Accessed 5-22-2018.

4. Zollinger H and Langhals H. Color Chemistry. Snythesis, Properties and Applications of Organic Dyes and Pigments. 3rd revised edition. *Angewandte Chemie International Edition*. 2004;43(40)

5. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients. College Park, MD: 2019. Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 3, 2019; received February 13, 2019).

6. Personal Care Products Council. 2017. Concentration of Use by FDA Product Category: Basic Red 76. Unpublished data submitted by Personal Care Products Council on December 13, 2017.

7. Thyssen JP, Sosted H, Uter W, et al. Self-testing for contact sensitization to hair dyes - scientific considerations and clinical concerns of an industry-led screening programme. *Contact Dermatitis*. 2012;66:300-311.

8. Goossens A. Self-testing for contact sensitization to hair dyes. *Contact Dermatitis*. 2012;66:299-299.

9. Coenraads PJ, Aberer W, Cristaudo A, et al. Allergy Alert Test for *p*-Phenylenediamine-Allergic Hair Dye Users. *Dermatitis*. 2018;29(5):250-257.

10. European Commission. Commision Regulation (EU) No 1197/2013 of 25 November 2013 amending Annex III to Regulation (EC) No 1223/2009 of the European Parliament and of the Council on cosmetic products. *Official Journal of the European Union*. 2013; https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:315:0034:0066:EN:PDF.

11. National Industrial Chemicals Notification and Assessment Scheme. Dyes that could release selected carcinogenic amines (listed on AICS): Human health tier II assessment. https://www.nicnas.gov.au/chemical-information/imap-assessments/imap-group-assessment-report?assessment_id=1418#cas-A_68391-30-0. Last Updated 2018. Date Accessed 6-1-2018.

12. Anonymous. 2005. Summary: Percutaneous penetration in vitro with Basic Red 76 (C008) in line with OECD TG 428. Report: R 0500455. Unpublished data submitted by Personal Care Products Council.

13. Anonymous. 2004. Summary: Acute oral toxicity with Basic Red 76 (C008) in line with OECD TG 423. Report: R0400806. Unpublished data submitted by Personal Care Products Council.

14. Anonymous. 2005. Summary: Sub-chronic oral systemic toxicity with Basic Red 76 (C008) in line with OECD TG 408. Report: R 0500465. Unpublished data submitted by Personal Care Products Council.

15. Anonymous. 2006. Summary: Prenatal developmental toxicity with Basic Red 76 (C008) in line with OECD TG 414. Report: R 0600049. Unpublished data submitted by Personal Care Products Council.

16. Anonymous. 2005. Summary: Micronucleus test in vitro with Basic Red 76 (C008) in line with OECD TG473. Report: R 0500330. Unpublished data submitted by Personal Care Products Council.

17. Anonymous. 2005. Summary: Micronucleus test *in vivo* with Basic Red 76 (C008) in line with OECD TG 474. Report: R 0500436. Unpublished data submitted by Personal Care Products Council.

18. Anonymous. 2004. Summary: Acute skin irritation with Basic Red 76 (C008) in line with OECD TG 404. Report: R 0400795. Unpublished data submitted by Personal Care Products Council.

19. Anonymous. 2004. Summary: Local lymph node assay (LLNA) with Basic Red 76 (C008) in line with OECD TG 429. Report: R 0400470. Unpublished data submitted by Personal Care Products Council.

20. Anonymous. 2004. Summary: Acute eye irritation with Basic Red 76 (C008) in line with OECD TG 405. Report: R 0400802. Unpublished data submitted by Personal Care Products Council.

21. Anonymous. 2005. Summary: Ames test with Basic Red 76 (C008) in line with OECD TG 471. Report: R 0500151. Unpublished data submitted by Personal Care Products Council.

22. Anonymous. 2005. Summary: Cell mutation test at TK locus (MLA) with Basic Red 76 (C008) in line with OECD TG 476. Report: R 0500184. Unpublished data submitted by Personal Care Products Council.

2019 VCRP Data

06A - Hair Dyes and Colors (all types requiring caution statements and patch tests)	**5**
06B - Hair Tints	**3**
06C - Hair Rinses (coloring)	**24**
06D - Hair Shampoos (coloring)	**11**
06H - Other Hair Coloring Preparation	**5**
08E - Nail Polish and Enamel	**2**

Concentration of Use by FDA Product Category – Basic Red 76

Product Category	Maximum Concentration of Use
Hair dyes and colors	0.057-0.35%
Hair tints	0.18%
Hair shampoos (coloring)	0.2%
Other hair coloring preparations	0.13%

Information collected in 2017

Table prepared December 13, 2017

Memorandum

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

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

DATE: November 28, 2018

SUBJECT: Draft Report: Safety Assessment of Basic Red 76 as Used in Cosmetics (draft prepared for the December 3-4, 2018 CIR Expert Panel Meeting)

The Council respectfully submits the following comments on the draft report, Safety Assessment of Basic Red 76 as Used in Cosmetics.

Key Issue

Carcinogenicity; Summary - The NICNAS hazard assessment does not belong in the Carcinogenicity section. If it is left in the report, who completed this hazard assessment and why it was completed should be made clear in the CIR report. This assessment was completed for the purposes of complying with labeling of the material, and not a consideration of the safe use of the material in consumer products. In which species were the tumors observed? The presentation of this hazard assessment also needs to be revised in the Summary. If NICNAS "provided" studies, they should be cited directly.

Additional Considerations

Cosmetic Use - The high concentration of PPD used in the self-test study was 2% not 0.2% as stated in the CIR report.

Toxicokinetics - NADH is generally considered a cofactor or coenzyme rather than an enzyme.

Dermal Penetration - Does the amount (1.44%, 0.046%, 0.0049%) in the stratum corneum, epidermis/dermis and receptor fluid really relate to the amount of "direct dye cream" as stated, or the amount of the Basic Red 76?

ADME - What was the duration of the urine and feces collection period in the dermal study (55.5%) in rats?

Genotoxicity - Please indicate which material was tested in the text.

Summary - What is meant by "bioavailable"?

Please identify the "test material" used in the rats study in which rats were dosed dermally with 0.2% including a radiolabeled compound.

1620 I. Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

It should be made clear that the 12 week study in rats was a single dose study. It would be more useful to state the effects that occurred at 200 mg/kg/day than to state that the NOAEL was <200 mg/kg/day.

As the time of treatment relative to gestation will in part determine the outcome, the time during gestation animals were treated should be stated in the Summary for all developmental toxicity studies.

It is not appropriate to state that "Mixed results were seen" in the genotoxicity studies. The Genotoxicity section says that the Basic Red 76 was generally not genotoxic. The only positive genotoxicity results were in two strains of *S. typhimurium* in one assay, and in an *in vitro* micronucleus assay in V79 cells at concentrations associated with test material precipitation.

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

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

DATE: January 22, 2019

SUBJECT: Tentative Report: Safety Assessment of Basic Red 76 as Used in Cosmetics (release date January 7, 2019)

The Council respectfully submits the following comments on the tentative report, Safety Assessment of Basic Red 76 as Used in Cosmetics.

Cosmetic Use - Please include the suggested conditions for hair-dye patch testing from reference 9, e.g., 45 minute open application of the hair dye after mixing with developer.

Dermal Penetration, Animal - Please revise: "Approximately 1.44%, 0.046%, and 0.0049% of the direct dye cream was present in the stratum corneum...". Rather than measuring the dye cream, they were measuring Basic Red 76 found in the dye cream.

Genotoxicity - It should be made clear that Basic Red 76 was negative in the chromosomal aberration assay in Chinese hamster V79 cells at a concentration of 106.3 mg/ml.

Carcinogenicity, Summary - NICNAS states that their assessment was based on third party summaries of studies, such as summaries found on the ECHA website. Therefore, it is not clear why the CIR report states: "according to several animal studies provided by the National Industrial Chemicals Notification and Assessment Scheme (NICNAS)". If NICNAS did not have the studies, how could they provide them to be cited in the CIR report? This should be changed to: "according to third party summaries of studies cited by NICNAS." It should also be stated that NICNAS did not have any specific evidence that Basic Red 76 is metabolized to *o*-anisidine.

Summary - Please correct "direct dye cream lotion" to "direct dye cream" as stated earlier in the report.

In addition to the concentration of Basic Red 76, please state the dose that was associated with adverse effects in the 12 week rat study (highlighted sentence).

It is not correct to include the genotoxicity study in mouse lymphoma cells in the sentence describing "mixed results". The results in mouse lymphoma cells were only negative.

Discussion - As Basic Red 76 is an ingredient used in hair dye products, please correct: "used as a rinse-off product" to "used in rinse-off products".

1620 L Street, N.W., Suite 1200 | Washington, D.C. 20036 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org