Amended Safety Assessment of Acid Violet 43 as Used in Cosmetics

Status: Draft Final Amended Report for Panel Review
Release Date: November 11, 2016
Panel Meeting Date: December 5-6, 2016
Memorandum

To: CIR Expert Panel Members and Liaisons
From: Monice M. Fiume
       Assistant Director/Senior Scientific Analyst
Date: November 11, 2016
Subject: Amended Safety Assessment of Acid Violet 43 as Used in Cosmetics

Enclosed is the draft Final Amended Report of the Safety Assessment of Acid Violet 43 as Used in Cosmetics (acidvi122016rep). At the June meeting, the Panel issued a Tentative Amended Report with the conclusion that Acid Violet 43 is safe in the present practices of use and concentration for use in hair dye formulations; this conclusion supersedes the conclusion published in 2001.

At the June meeting, the Panel considered data that have become available since the original safety assessment was issued. In that original assessment (acidvi122016prev), most of the data pertained to the certified color Ext. D&C Violet No. 2; therefore the Panel issued a lengthy conclusion that specifically defined impurities specifications. The new information that was reviewed by the Panel in June included test data on both Acid Violet 43 and Ext. D&C Violet No. 2. Subsequently, the Panel determined that the new information could be used to assess the safety of Acid Violet 43, re-opening the report to remove the impurity specifications from the conclusion.

The Panel should review the Discussion to ensure it adequately reflects the opinions of the Panel and be prepared to issue a Final Amended Report.
*If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.*
ACID VIOLET 43

2001: Original Report Published
The Panel published a safety assessment with the conclusion that Acid Violet 43 is safe for use in hair dye formulations when free of impurities except for the following: ≤18% volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); ≤0.4% water-insoluble matter; ≤0.2% 1-hydroxy-9,10-anthracenedione; ≤0.2% 1,4-dihydroxy-9,10-anthracenedione; ≤0.1% p-toluidine; ≤0.2% p-toluidine sulfonic acids, sodium salts; ≤1% subsidiary colors; ≤20 ppm lead (as Pb); ≤3 ppm arsenic (as As); ≤1 ppm mercury as (Hg); and ≥80% total color. These are the specifications for Ext. D&C Violet No. 2, which is a certified colorant, and these specifications were included in the Panel’s conclusion for Acid Violet 43 because Ext. D&C Violet No. 2 was tested in the safety studies described in the original safety assessment.

June 2016: Re-Review
A re-review document was submitted to the Panel for review. This document included data on Acid Violet 43 and new data on Ext. D&C Violet No. 2. The Panel opened the re-review to eliminate the impurity specifications in the conclusion because of the new data on Acid Violet 43 that are now included in the report.

Dec 2016: Final Amended Report
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## Acid Violet 43 RR

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**PubMed Search Strategy – 4/20/16**
(4430-18-6[EC/RN Number]) OR (ACID AND VIOLET AND 43)

**SciFinder – 4/20/16**
4430-18-6; refined by document type
RE-REVIEW - ACID VIOLET 43
FULL PANEL – June 7, 2016

DR. BELSITO: So we again thought we needed to reopen this because with the original, since we had no information on the acid violet that was used in hair products, we restricted impurities to the food grade level. However, now we have a 13 week oral, repro, genotox, sensitization, and irritation on the less pure material. So we thought we could reopen to lift the very stringent restrictions that we had put in to the original conclusion.

DR. MARKS: Second.

DR. BERGFELD: Immediate change in plan. It's been seconded to reopen this ingredient, which is the acid violet 43. Any comments? Ron Shank is looking.

DR. SHANK: I'm trying to find the exact wording.

DR. BELSITO: The original conclusion?

DR. SNYDER: And put all the limits on all the impurities.

DR. LIEBLER: It's on page 2, Ron Shank.

DR. SHANK: Pardon me?

DR. LIEBLER: It's on page 2 from (inaudible) memo. And if you look in the first paragraph you'll see the very lengthy conclusion of all the restrictions based on what Don has already stated. And you want to simplify it and move forward to safe.

DR. BERGFELD: All right.

DR. BELSITO: WE don't need the restrictions anymore.

DR. BERGFELD: Because the uncertified product has tested. Okay or not? All right. I'm going to call the question. All those to reopen please indicate. Yes. unanimous.

Belsito Team – June 6, 2016

DR. BELSITO: Okay, Acid Violet 43. This is Monice (music starts playing). Whoa, we're getting psychedelic here.

(Laughter) This is Acid Violet (inaudible).

DR. BELSITO: Oh, I see.

DR. LIEBLER: Once (inaudible) at (inaudible).

DR. BELSITO: Yeah, I guess. That was you, Dan?

DR. LIEBLER: No, that was YouTube.

DR. BELSITO: Okay. (Laughter) Okay, so Acid Violet 43. Previously we reviewed the conclusion that it was safe for use in hair dye formulations when free of certain impurities. There's been a slight increase in use and concentration in hair coloring formulations.

I think the most important thing about this is back in 2001 when we looked at it, we had data only for the certified colorant which was Violet 2 and we based our decision on the idea that the hair colorant, Acid Violet 43, would match that same purification level or impurity levels. And now we have a bulk of data for the actual presumably less pure Acid Violet 43, which is also pretty clear data. I mean and there's nothing coming up. So I guess in my mind, the only question I had is do we need to continue to restrict the hair dye Acid Violet 43 to the same stringent impurity requirements that we had originally limited it based upon the fact that we had data only for Violet 2?

And so we have a 13 week oral, repro, genotox, and sensitization and irritation on the less pure material. And they were all clean.

DR. LIEBLER: Yeah, I thought we could reopen this to simplify the original conclusion because we've got enough data now to define that Acid Violet 43.

DR. BERGFELD: Is it going to change it the conclusion at all?

DR. LIEBLER: Other than to remove all that excess verbiage with the specific restrictions on the amounts of the different impurities.
DR. BELSITO: Yeah, our conclusion really had a significant... I mean it was that in the conclusion or was it in the discussion because there was

MS. FIUME: It's in the introduction.

DR. BELSITO: Right,

MS. FIUME: The

DR. BELSITO: No, no, but the

MS. FIUME: Oh, it was in the conclusion.

DR. BELSITO: It was in the so the restrictions on impurities were in the conclusion.

DR. LIEBLER: I'd never seen one that had that much stuff in it.

MS. FIUME: Well, you haven't looked at that.

DR. LIEBLER: Really? Okay, I haven't.

MS. FIUME: AHAs.

DR. LEIBLER: I haven't seen the ancient texts, okay.

DR. BELSITO: Yeah, so I mean if those restrictions are in the conclusion we need to reopen it to remove them.

DR. SNYDER: I had a question on other use, onetime use. The first paragraph says that Acid Violet is reported used in oxidative and direct hair coloring products.

DR. BELSITO: Yes.

DR. SNYDER: And then down below, two more paragraphs, the paragraph that starts in 2013 the SCCS concluded that the use of Acid Violet as a non oxidative hair dye. So are we using the direct, indirect for the hair dye designation and the oxidative, non oxidative or

DR. BELSITO: Yeah, indirect is non oxidative?

DR. SNYDER: Correct.

DR. BELSITO: And direct is oxidative.

DR. SNYDER: Right, but that kind of contradicts, right? It says it's used in oxidative and direct above, and then down here it says in this paragraph, it says twice. It says in non oxidative hair dye. So which is it?

DR. EISENMANN: It's a non oxidative, but it could be in an oxidative, like to modify the color is my understanding. So occasionally you might have other components in as part of an oxidative hair dye that doesn't isn't it part of the process of the oxidation process? It's like a modifier. So it could be used in the final formulations, but itself is not an oxidative.

DR. SNYDER: So we should more actively say it's used in oxidative and non oxidative hair dyes, formulations.

DR. EISENMANN: It could be, but itself, it's not a non it's a non oxidative. It doesn't take part in those reactions.

DR. BELSITO: And non oxidative hair dyes are direct hair dyes, right? The term direct refers to non oxidative. So they directly stain the keratin of the hair.

DR. EISENMANN: That's my understanding.

DR. BELSITO: Right. So I think we need to reopen simply to change the conclusion and get rid of the restrictions on impurities that we had previously.

DR. SNYDER: Agree.

DR. BERGFELD: Could I just ask one question? Do you think that you understand the chemistry of the two different dyes? How close are they?

DR. BELSITO: Well, they're the same.

DR. BERGFELD: They're same.

DR. BELSITO: I mean the dyes are the same. The difference was the degree of purity of the actual material that was supplied. But again, we have a I mean they're brief discontinuous use. They're hair dyes only. We have a 13 week oral on the less pure or repro or genotox, sensitization and irritation. So I think that we really have everything that we need to cover the impurities aspect of these.

I guess, you know, the only other issue that this always brings up for me is when you're dealing with a non oxidative direct hair dye, are those exempt based upon that 1932 coal tar exemption or is only the oxidative hair dyes that are exempt for I mean this was clean for sensitization and irritation. I just raise it for future ones we may look at. Is it only the oxidative hair
dyes that are exempt? I mean because I this is presumably it's an anthraquinone and it's a coal tar derivative. So does that regulation exempt all of them?

MS. FIUME: I'm not sure. I when we were discussing getting ready for the meeting; CIR staff and Carol, you may be able to help us, we actually got ourselves confused with certified versus non certified and what exactly it means and if we can get a definition. I was trying to look at the dictionary and it said those that are exempt are the coal tar hair dyes, but I wasn't sure if it was non certified, direct, or non direct. So can you give us a little information on that on how it works?

DR. SADRIEH: I mean the coal tar hair dyes are the only ones that are exempt. Certified I think is for batch certification. As for color additives it's completely different. It doesn't have anything to do with the coal tar hair dyes not from my understanding.

DR. EISENMANN: Except there's a few crossovers.

DR. SADRIEH: Yes, yes.

DR. EISENMANN: Are they used as (inaudible) hair dye

DR. SADRIEH: By the

DR. EISENMANN: and the certified color, this is one of them. So

MS. FIUME: So non certified would be a hair colorant with a cosmetic use. And certified is a colorant that has

DR. EISENMANN: Well you'd have to look at the regulation to see what use is it permitted for.

MS. FIUME: Right.

DR. BERGFELD: What's your opinion on the difference between these two dye groups?

DR. EISENMANN: I haven't looked at the information that closely, but I wasn't necessarily that I wasn't convinced that of the material that was tested for SCC European Opinion. I didn't know if they'd measured the what the FDA measures to certify the ingredient. So I don't know how they actually differ that well because I don't know if the analysis was the same.

MS. SADRIEH: Right, the certification is is you need to look for contaminants. That there are certain specifications that have to be met. That's what the certification looks at. And, you know, they will be certifying not just cosmetic, you know, color additives, but other types of color additives used. So it depends on the use of it. I think for cosmetic purposes coal tar hair dyes technically will be considered exempt. So this might be certified, but it's

MS. FIUME: Exempt.

DR. SADRIEH: for the use.

MS. FIUME: Exactly.

DR. BERGFELD: Yeah, it's my opinion, and I'm not a chemist, that these two are pretty much alike. (Laughs) And

DR. EISENMANN: I wasn't able to tell if they you know, if what they look for in the I mean, you have specification or what the material is that was tested for those studies, but I'm not sure how if it differs from the if they because if they act they may not have measured what the FDA is required to measure to for certification.

DR. SADRIEH: I mean, I don't know if this particular, you know, ingredient, what the things would be, but normally they would look at, you know, aromatic (inaudible) and heavy metals and, you know, other types of contaminants that would be potentially present. And so depending on the actual certified color additive, they would look for different types of things that are part of the specifications.

DR. LIEBLER: Just to clarify one thing. I've heard someone mention a couple times about the possible structural differences. The 43 and 2 are the same structure.

DR. BERGFELD: Right.

DR. LIEBLER: It's just a different degree of analysis of possible impurities.

DR. BERGFELD: So I'm not sure where we're going with reopening. I guess that's where I am. If they're similar you could cover in your discussion the differences

DR. BELSITO: But our conclusions specifically regulated it and which levels of impurities which do not need to be regulated anymore. So it is a different conclusion. I mean in the conclusion we went on to say it has to be this degree of purity

DR. SNYDER: Against specific percentages as the of the impurities in the (inaudible).

DR. BELSITO: And now we have

DR. BERGFELD: But this is including two ingredients that are similar in one report. Now we're adding the certified
DR. BELSITO: No, no, no, we what we had is a dye. One dye that's used is a food and colorant with a very pure specification. And we had no information on what exactly was used as the hair dye Acid Violet. And so we said, okay, we'll go safe as used as long as the Acid Violet that's used in hair dye matches the same purity as it does for food and colorants. And now, the bulk of the data we have here is on a relatively impure, probably what is actually put into the hair dye, and so we don't need and on top of that, we had very good tox data to cover brief discontinuous use. We had 13 weeks. We had oral. We had genotox. We had repro. We had sensitization and irritation. So I think we don't need those restrictions in the conclusion anymore. So we have to reopen to change the conclusion. Safe as used for brief discontinuous use without four sentences full of restrictions. It's a new conclusion.

DR. BERGFELD: I'm just going to look at the old.

DR. BELSITO: Because otherwise we're telling industry, okay, we have the data on what's probably used as a hair dye, but even though we have that data and it's clean, you still have to use a colorant that meets the food and safety levels. And I don't think, in fact, that we have the data that suggests it does not have to be that pure in a hair dye. Just knock over everything there, Paul.

DR. SADRIEH: So I just want to conclude that basically because it is exempt, the Violet 43 basically, it's it can contain impurities that would not be allowed in the certified piece.

DR. BELSITO: Right.

DR. SADRIEH: D and C Violet No. 2.

DR. BELSITO: Right.

DR. SADRIEH: Right.

DR. SNYDER: The new report will incorporate the impurities that we now know and the (inaudible) so we can

DR. BELSITO: Right.

DR. SNYDER: It's based on that known data. This is all a report (inaudible).

DR. BERGFELD: I understand changing the conclusion, and so, fine. But I think in your discussion you're going to have to discuss this, the differences between the two.

DR. BELSITO: Yes, and why the conclusion was changed.

DR. BERGFELD: Yeah.

DR. BELSITO: And as Paul pointed out, the impurities we expect to be in the hair dye are the impurities that we're told are in Acid Violet 43 and the new data that we've received were the genotox, repro, et cetera.

DR. BERGFELD: Well I gather the only closing remark, as my husband says I always have the last word. If one industry gives you the hair dye ingredients and say there's another industry that makes it and it's an uncertified hair dye, you're not unknowledgeable about the differences between the two product lines coming from two different companies.

DR. BELSITO: We have that problem all the time.

DR. BERGFELD: I know because

DR. BELSITO: And we simply say that we assume that what's being provided is similar to what we looked at.

DR. BERGFELD: Yeah, I'd like to see that discussion as well.

DR. BELSITO: No, that

DR. BERGFELD: Okay.

DR. BELSITO: I think we said that that would be in the discussion.

DR. SNYDER: I mean some of them, they give a range of how pure it is. Like 50 percent (inaudible) it went down to 50 percent pure or something. Or we could I think that can be in the discussion.

DR. BERGFELD: Mike?

MR. BEST: Yes, a quick question. I think that (inaudible) that also relates back to the persulfates. I was just curious I mean, if you had sort of a range of what brief discontinuous use is? So like with the persulfates and there's all these new clinical studies where clearly, occupationally, that seems to be a problematic as an irritant and sensitizer. I mean, if you are a consumer using one of these products every month for 20 years, does that become continuous use? You know what I mean? Like, what's the interval that you're thinking of when you say discontinuous?

DR. BELSITO: What we mean by that is that the product we're not talking about the longevity in terms of doing it every six weeks for the rest of your life.

MR. BEST: Right.
DR. BELSITO: We're talking about short term contact that's followed by rinsing.

DR. SNYDER: But each individual exposure is brief.

MR. BEST: So the individual exposure stacked up over time you don't see as problematic? It's the life the contact exposure, the individual one. Okay.

DR. BELSITO: Well, I mean it could be problematic if there's significant absorption of the material and how frequently you might use it, then those would all come into play.

MR. BEST: Okay, thank you.

MS. FIUME: So Dr. Belsito, can I clarify then on page 13, PDF page 13, where we have the reference to the coal tar hair dyes. That does stay in this report?

DR. BELSITO: I'm gathering. I mean it you know, the this is

MS. FIUME: It should.

DR. BELSITO: an anthraquinone, right?

DR. SNYDER: Yes, it is.

DR. BELSITO: So it is a coal tar derivative.

DR. SNYDER: Yep.

MS. FIUME: (inaudible)

DR. BELSITO: So I just always raise the question because I'm never quite sure what that excludes, but I guess as long as it's a coal tar derivative, whether it's oxidative or non oxidative, it's exempt. Anything else? Okay.

Marks Team – June 6, 2016

DR. MARKS: Okay. Any other comments? If not, we'll move on to acid violet 43 aka what was the other name for it?

MS. FIUME: D&C violet #2. That's the certified name.

DR. MARKS: So so this is a re review of a safety assessment with acid violet 43 in 2001. The panel published the safety assessment with a conclusion that acid violet 43 is safe for use in hair dye formulation when free of impurities except for the following. And then there was a whole laundry list of except for the following, Monice, that's on May 13th memo. Do you want me to read all those? Less than 18 percent volatile matter at 135 degrees Centigrade et cetera. I'll assume it will be captured.

MS. FIUME: It is and it's in the introduction. And what that refers to is the specifications for the certified color.

DR. MARKS: These impurities were included in the panel's conclusion for acid violet 43 because the what's the Ext stand for? D&C violet #2 was tested in the safety studies described in the original assessment. Acid violet 43 has the same chemical structure as what's the Ext stand for?

DR. LORETZ: Externally.

MS. FIUME: I've only seen it written as Ext. I've never seen it written differently.

DR. MARKS: Ext D&C violet #2, but is not a certified color and could not have impurities that are not allowed in the certified color. So and then we have (inaudible) you mention in your memo on the let's see, two, three fourth paragraph, that the European Commission, a Scientific Committee on Consumer Safety, concluded that (inaudible) maximum head on concentration of 0.5 percent does not pose a risk for health. Ron, Ron, and Tom don't reopen?

DR. SHANK: Correct.

DR. SLAGA: Correct.

DR. MARKS: Okay.

DR. HILL: So was the elected conclusion is now?

DR. MARKS: Oh, it's the same conclusion as the original one.

DR. HILL: I know. I'm looking to remind myself what.

DR. SHANK: Safe for use in hair dye formulations.

DR. HILL: For the acid violet?

DR. SHANK: Yes.

DR. HILL: Okay. And I agree.
DR. MARKS: When free of all impurities.
DR. SHANK: When free of all that stuff.
DR. HILL: Well, okay, so, you know, my
DR. SHANK: (Inaudible).
DR. HILL: no, no. It raised an interesting issue, because it what I put was what did I put is expected for a compound of this structure, especially noting the sulfonate, dermal penetration is very low and then coupled with low usage concentrations, there should be minimal concern. Acid violet 43 was shot down versus Ext D&C #2 over concerns of additional purities. So, in fact, it's a toxicology resulting from the presence of impurities in the AV43 that are not present in the D&C #2.
So the impurities were there. They performed the toxicology studies that caused it to be shot down and we don't normally review an ingredient based you know, I mean we take into account impurities, but this is strange.
I still agree with do not reopen and I agree with the conclusion, other than it's crazy to have those limits in there in that way.
MS. FIUME: Dr. Hill, I think what the difference is is the original report all of the data were on the certified color
DR. HILL: Yes.
MS. FIUME: Ext D&C violet #2. And so the purity of that they know it's above 80 percent. Whereas in the hair dye that's not certified, the impurity level is from 54 to 60 percent.
DR. HILL: Yeah. I got it. But what's weird about this is I mean I realize we are taking impurities into account and I'm always sitting here saying give me more information on the impurities. But you never in the acid violet 43, that can vary from lot to lot to lot to lot. In fact, it could even exceed the D&C #2. And so it just feels strange. I read every line of the transcript from the previous meetings and I read the discussion and I read all the pertinent sections of the old report and I it seems strange to say, all right, well, we will go with I don't know.
It just bothered me at a level that I thought we ought to be able to pull out those specifications and just leave it safe and have the discussion address the whole idea of the impurities. Because because the acid violet 43 is specific to a manufacturing process, I guess, that's a little less stringent on the purification end. But, I mean, we see this all the time in numerous ingredients, honestly. But we're not going to reopen, right? So we wouldn't we wouldn't change the conclusion anyway.
DR. MARKS: Not reopen.
DR. HILL: It just
DR. HILL: Agree.
MINUTES FROM ORIGINAL DELIBERATIONS - ACID VIOLET 43

April 3-4, 1997 – informal data request

Dr. Belsito noted that additional data on this ingredient, largely derived from FDA's files on External D&C Violet No. 2 (FDA's certified form of Acid Violet 43) were received. However, he stressed that the Panel is not reviewing the safety of Ext. D&C Violet No. 2, but, the safety of Acid Violet 43, as it is used in hair dyes.

Dr. Belsito also said that his Team determined that the available data remain insufficient for evaluating the safety of Acid Violet 43 in cosmetics, and that the following data are needed: (1) 1997 concentration of use data and (2) absorption under conditions of use; if absorption occurs, then a 28-day dermal toxicity study as well as a reproductive toxicity study will be needed.

Dr. Schroeter noted that his Team determined that a 28-day dermal toxicity study would not be needed, after considering the negative short-term dermal toxicity study (guinea pigs) on a hydrophilic ointment containing 0.1 or 1.0% Acid Violet 43. Applications were made over a three-week period in this study. Dr. Schroeter's Team also requested reproductive and developmental toxicity data (i.e., if absorption occurs), as well as a skin irritation and sensitization study.

Dr. Belsito recalled that his Team did not request skin irritation and sensitization data because hair dye products containing Acid Violet 43 carry a warning statement and patch test instructions. With this in mind, Dr. Belsito said that skin irritation and sensitization data, if provided, would be deemed irrelevant to the safety assessment of Acid Violet 43.

Dr. Shank noted that Acid Violet 43 is used in many products, other than hair dyes. Dr. Belsito said that Acid Violet 43 is used in other types of products as Ext. D&C Violet No. 2.

Dr. Shank said that if this is the case, then Table 1 (FDA product formulation data) of the Draft Report needs to be revised. Dr. Belsito said that use frequencies for products containing Ext. D&C Violet No. 2 should be deleted from Table 1. Furthermore, he said the product category, Deodorants (Underam) should be starred as an illegal use of Acid Violet 43.

Dr. Shank also commented that, based on his understanding, the Panel has been asked to evaluate the safety of Acid Violet 43 for use only in hair dye products. Dr. McEwen noted that the Panel is reviewing the safety of an ingredient that can be used in a form that is certified by FDA for use in cosmetics, and, thus, the Panel is precluded from evaluating the safety of Ext. D&C Violet No. 2 (certified color) because FDA has ruled on its safety. He then recalled that the other use can only be, as the law is written for colorants, in hair dyes (those with the warnings and directions for use). Therefore, he said that anyone who is using Acid Violet 43 in a product other than a hair dye (with warning statement) is doing so in contravention of the Food, Drug, and Cosmetics Act.

Dr. Bergfeld understood Dr. McEwen's comments to mean that the cosmetic use of Acid Violet 43 can be isolated to use in hair coloring. With this in mind, she said that all information concerning cosmetic uses of Ext. D&C Violet No. 2 should be deleted from the Draft Report.

Taking into consideration the caution statement and patch test instructions associated with hair dyes containing Acid Violet 43 and that hair dyes are rinse-off products, Dr. Bergfeld wanted to know whether a reproductive and developmental toxicity study will still be needed if Acid Violet 43 is found to be percutaneously absorbed.

Drs. Schroeter and Belsito agreed that reproductive and developmental toxicity data will still be needed.

Ms. Fiume recalled that reproductive and developmental toxicity data were not mentioned in the informal data request issued in Teams at the September 1996 Panel meeting, and suggested that the list of data needs be revised and reissued.

Dr. McEwen noted that there was no indication of systemic toxicity in long-term dermal toxicity studies. With this in mind, he wanted to know why reproductive and developmental toxicity data should be included in the Panel's data requests on Acid Violet 43.

Dr. Belsito noted that the short-term and subchronic dermal toxicity studies in the Draft Report are on Ext. D&C Violet No. 2.

Dr. McEwen asked Dr. Bailey to comment on the number of petitions for certification of Ext. D & C Violet No. 2 that had been turned down by FDA. Dr. McEwen also said that the fact that Acid Violet 43 has not been certified does not necessarily mean that it is different from the certified dye (Ext. D&C Violet No. 2).

Dr. Bailey said that based on his experience in dealing with certified versus non-certified batches, there have been considerable differences between technical grade versus food and drug or cosmetic grades. With respect to petitions being turned down, he said that he does not think FDA has turned down any petitions for Ext. D&C Violet No. 2. Deliberations on petitions were held, data requirements were established, and, eventually, the data provided were sufficient for supporting safety. Dr. Bailey said that he is uncomfortable with the assumption that Acid Violet 43 is equivalent to Ext. D&C Violet No. 2.
Dr. Bailey also said that one can always say that Acid Violet 43 is safe for use as a hair dye, and, if it is Ext. D & C Violet No. 2, there is nothing to prohibit that.

Dr. McEwen said that he would be against trying to make hair dyes into certified colors.

Dr. Bailey clarified that Ext. D&C Violet No. 2 is the regulatory name for the color, and that it can only be used after it has been analyzed and shown to be of a certain chemical composition and purity.

Dr. Carlton asked Dr. Bailey if his comments on the certification of colors refer to cosmetic use.

Dr. Bailey said that his comments are applicable to externally applied cosmetics.

Dr. Andersen emphasized that an FDA-regulated cosmetic ingredient (e.g. Ext. D&C Violet No. 2) is exempt from the CIR review process, regardless of its use. He also said that Acid Violet 43, as an uncertified color, only has legal use in hair dyes, and, as such, the Panel has to review its potential use in hair dyes. He noted that uses of the uncertified color Acid Violet 43 are being reported, as indicated in Table 1 of the Draft Report.

Because the Panel's initial informal data request on Acid Violet 43 was revised at the present meeting, the Panel unanimously approved the issuance of a second informal request with the following data needs: (1) 1997 concentrations of use and (2) a dermal absorption study on uncertified Acid Violet 43; if absorbed, then a 28-day dermal toxicity study and a reproductive toxicity study are needed.

Dr. Bergfeld said that the report discussion that eventually will be developed should contain the Panel's discussion on certified vs. uncertified colors. The discussion section of the report will provide the basis for any additional data needed for completion of the Panel's safety assessment of Acid Violet 43.

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**June 5-6, 1997 – IDA was issued**

Dr. Belsito noted that the Draft Report on Acid Violet 43 was reviewed at the April 3-4, 1997 Panel meeting, where informal data requests were issued during the closed session (in Teams). The Belsito Team determined that the following data are needed for completion of the safety assessment on Acid Violet 43: (1) Concentration of use and (2) Dermal absorption under conditions of use; if absorbed, 28-day dermal toxicity data and dermal reproductive/developmental toxicity data are needed. It was requested that the testing be performed on Acid Violet 43, not the certified color.

The Panel voted unanimously in favor of issuing an Insufficient Data Announcement with the following data requests:

(1) Concentration of use

(2) Dermal absorption under conditions of use; if absorbed, 28-day dermal toxicity data and dermal reproductive/developmental toxicity data are needed

*The testing is to be performed on Acid Violet 43, not Ext. D&C Violet No. 2.

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**December 8-9, 1997 – report was tabled**

Dr. Belsito said that the basic problem concerning this ingredient is determining which data in the CIR report are on Acid Violet 43 versus which are on Ext. D&C Violet No.2. Ext. D&C Violet No.2 is certified and, therefore, a potentially pure dye. Dr. Belsito also said that because of this problem and his Team's belief that some of the more critical data, particularly the dermal carcinogenicity data on Ext. D&C Violet No. 2, were on Ext. D&C Violet No.2, his Team determined that the data in the CIR report on Acid Violet 43 are insufficient for evaluating the safety of this chemical. Dr. Belsito noted that his Team had determined that the following data on Acid Violet 43 are needed: (1) Current concentration of use data and (2) Dermal absorption under conditions of use; if absorbed, 28-day dermal toxicity data and dermal reproductive/developmental toxicity data are needed.

Dr. Bergfeld asked if clarification on Acid Violet 43 and Ext. D&C Violet No. 2 is needed.

Dr. Belsito said that if the Panel can receive clarification on the two dyes, the dermal carcinogenicity study that was performed over a period of 107 weeks might be useful.
Dr. McEwen said that it would be useful to know the difference between the dye that can be certified and the one that cannot be certified, the magnitude of that difference, and where that difference generally lies. He said that this information could be requested from those concerned with color certification as well as the manufacturers.

Dr. Bailey said that the certified Ext. D&C Violet No. 2 has limits on the composition of the impurities, the main coloring component, and other components of the color. These include components such as residues of heavy metals, moisture, the purity of the color etc., and specifications for residues of paratoluidine. Dr. Bailey said that he would expect that the uncertified color would be of a lesser degree of purity. He also said that the question of differences in toxicity between Acid Violet 43 and Ext. D&C Violet No.2 may be valid, depending on the magnitude of differences in composition.

Dr. Andersen asked if FDA had turned down any request for Ext. D&C Violet No. 2 certification and why.

Dr. Bailey said that FDA could provide this information. However, he said that he is not sure of how useful this information would be to CIR, because the colors that are prepared and submitted for certification are generally of a purer grade. Dr. Bailey said that determining which grades are being used in other countries (where there is no certification requirement) may be more relevant.

The Panel voted unanimously in favor of tabling the report on Acid Violet 43.

Dr. Bergfeld said that the report on Acid Violet 43 is being tabled with the intention of clarifying exactly what these ingredients, Acid Violet 43 and Ext. D&C Violet No. 2, are and, possibly, the impurities that are present.

Dr. McEwen said that the issues raised may best be handled by obtaining dye specifications and incorporating this information into the current document.

March 19-20, 1998 – Tentative Report issued

Dr. Belsito noted that for the past several meetings, the Panel has struggled with the fact that much of the information in the CIR report is on External D&C Violet No. 2, the authorized chemical for coloring cosmetics, versus the hair dye ingredient, Acid Violet 43. Thus, the Panel asked for clarification of the exact specifications for Ext. D&C Violet No. 2, and past reasons why any batches of this chemical were classified as failing to meet these specifications. Dr. Belsito said that in response to this request, Dr. Bailey provided the Panel with FDA's criteria for Ext. D&C Violet No. 2. He also provided information indicating that one of the reasons why some batches have not been certified is because the chemical paratoluidine (which is not supposed to be present in amounts > 0.1%) was present at concentrations > 0.1%. With this in mind, Dr. Belsito suggested that the Panel conclude that Acid Violet 43 is safe for use as a hair colorant, as long as it meets the specifications that have been determined for Ext. D&C Violet No. 2. He noted that paratoluidine can cause problems.

Dr. Bergfeld confirmed with Dr. Belsito that the specifications referred to will be included in the report discussion.

Dr. McEwen noted that this approach is new to him. He thought that the approach that was discussed before, and decided against, was that Acid Violet 43 would be considered safe as long as it is Ext. D&C Violet No. 2. This means that Acid Violet 43 would have to be certified by FDA. Dr. McEwen said that there is nothing wrong with the Panel deciding, as it has done in the past, that there have to be certain specifications for Acid Violet 43.

Dr. Bailey said that the available data is ample in supporting a position that Ext. D&C Violet No. 2 is safe when used conservatively. He also said that if the Panel wants to impose specifications, he thinks that this is a very reasonable approach to take. Dr. Bailey said that Acid Violet 43 does not necessarily have to be certified, but it does have to meet individual specifications.

Time was allowed for the Panel to review FDA's specifications for Ext. D&C Violet No. 2 that were made available at this meeting.

Dr. Andersen recommended that the specifications be included in the report conclusion. In other words, it should be stated that Acid Violet 43 is safe for use in cosmetics, if it meets certain specifications (which will be listed). He said that Ext. D&C Violet No. 2. should not be mentioned in the report conclusion.

Dr. McEwen said that it should be stated that Acid Violet 43 is safe for use as a hair dye.

Ms. Fise said that the report conclusion should be helpful to those reading it, and suggested that the CFR (Code of Federal Regulations) should be cited, that is, the CFR reference to specifications on Ext. D&C Violet No. 2. Dr. McEwen said that the pertinent information from the CFR citation should be included in the report. In so doing, there would be no need for the reader to consult another document for information that is necessary. Dr. McEwen agreed with the suggestion that the specifications be included in the report discussion.

Dr. Schroeter said that the specifications need to be introduced somewhere in the text prior to inclusion in the report discussion.
Dr. Carlton favored including the specifications in the report conclusion.

Dr. Belsito suggested that the specifications should also be included in the report discussion. He also said that it should be pointed out in the discussion that the Panel does not have data on Acid Violet 43, but has a considerable amount of data on Ext. D&C Violet No. 2. With this in mind, he said that it should also be stated that it is possible for the Panel to arrive at a conclusion on the safety of Acid Violet 43 because, clearly, the dye Ext. D&C Violet No. 2 (i.e., Acid Violet 43 that has been certified by FDA) is safe. Dr. Belsito also suggested that the specifications should be included in the report conclusion.

The Panel voted in favor of issuing a Tentative Report with the following conclusion: The CIR Expert Panel concludes that Acid Violet 43 is safe for use in hair dye formulations when free of impurities, except for the following:

- 18% volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts);
- 0.4% water-insoluble matter;
- 0.2% 1-hydroxy-9,10-anthracenedione;
- 0.2% 1,4-dihydroxy-9,10-anthracenedione;
- 0.1% p-toluidine;
- 0.2% p-toluidine sulfonic acids, sodium salts;
- 1% subsidiary colors;
- 20 ppm lead (as Pb);
- 3 ppm arsenic (as As);
- 1 ppm mercury (as Hg); and
- 80% total color.

Four Panel members voted in favor of the conclusion, and there was one abstention (Dr. Shank).

Dr. Bergfeld noted that the conclusion was approved with the understanding that the specific citation and specifications will be included in the Chemistry section of the report and will be referenced and discussed in the report discussion. She said that the Panel's reason for including the specifications on Ext. D&C Violet No. 2 will also be included in the report discussion.

**Sept 11, 2008 – Final Report issued**

Dr. Belsito stated that sufficient data are available for arriving at a conclusion on the safety of Acid Violet 43 (hair dye), but that most of the data are on Ext. Violet No. 2 (a certified dye). He noted that Acid Violet 43 and Ext. Violet No. 2 have the same chemical structure; however, Acid Violet 43 is not certified and could contain impurities that are not allowed in Ext. Violet No. 2. With this in mind, Dr. Belsito said that his Team agreed to establish restrictions on impurities in Acid Violet 43.

The Panel voted unanimously in favor of issuing a Final Report on Acid Violet 43 with the following conclusion: The CIR Expert Panel concludes that Acid Violet 43 is safe for use in hair dye formulations when free of impurities except for the following:

- 18% volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts);
- 0.4% water-insoluble matter;
- 0.2% 1-hydroxy-9,10-anthracenedione;
- 0.2% 1,4-dihydroxy-9,10-anthracenedione;
- 0.1% p-toluidine;
- 0.2% p-toluidine sulfonic acids, sodium salts;
- 1% subsidiary colors;
- 20 ppm lead (as Pb);
- 3 ppm arsenic (as As);
- 1 ppm mercury (as Hg); and
- 80% total color.

Dr. McEwen noted that the origin of the limitations stated above is not included in the report discussion, and recommended inclusion of this information.
Amended Safety Assessment of Acid Violet 43 as Used in Cosmetics

Status: Draft Final Amended Report for Panel Review
Release Date: November 11, 2016
Panel Meeting Date: December 5-6, 2016

The 2016 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This safety assessment was prepared by Monice M. Fiume, Assistant Director/Senior Scientific Analyst.
ABSTRACT
The Cosmetic Ingredient Review (CIR) Expert Panel (Panel) re-opened the safety of Acid Violet 43, a cosmetic ingredient that is an anthraquinone dye reported to function in cosmetics as a colorant. This colorant has the same chemical structure as Ext. D&C Violet No. 2, which is a certified colorant; however, Acid Violet 43 is not a certified color and it could have impurities that are not allowed in the certified color. The Panel reviewed relevant new data related to this ingredient, and concluded that Acid Violet 43 is safe in the present practices of use and concentration for use in hair dye formulations. This conclusion supersedes the previous conclusion for Acid Violet 43 that included impurity specifications indicated for the certified color.

INTRODUCTION
In 2001, the CIR Expert Panel published a safety assessment with the conclusion that Acid Violet 43 is safe for use in hair dye formulations when free of impurities except for the following: ≤18% volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); ≤0.4% water-insoluble matter; ≤0.2% 1-hydroxy-9,10-anthracenedione; ≤0.2% 1,4-dihydroxy-9,10-anthracenedione; ≤0.1% p-toluidine; ≤0.2% p-toluidine sulfonic acids, sodium salts; ≤1% subsidiary colors; ≤20 ppm lead (as Pb); ≤3 ppm arsenic (as As); ≤1 ppm mercury (as Hg); and ≥80% total color.1 These restrictions refer to the specifications for Ext. D&C Violet No. 2. Acid Violet 43 has the same chemical structure as Ext. D&C Violet No. 2, but it is not a certified color and it could have impurities that are not allowed in the certified color. These specifications were included in the Panel’s original conclusion on Acid Violet 43 because the safety test data included in the original assessment pertained to Ext. D&C Violet No. 2.

In accordance with its Procedures, CIR evaluates the conclusions of previously-issued reports every 15 years to determine whether the conclusion should be reaffirmed. During the literature search was conducted for the re-review, an updated European Commission (EC) opinion was discovered. Because the EC Scientific Committee on Consumer Safety (SCCS) Opinion on Acid Violet 43 included new data on Acid Violet 43, the Panel conducted a re-review.2 Excerpts from the summary of the 2001 report are disseminated throughout the text of this re-review document, as appropriate, and are identified by italicized text; as stated above, the toxicity data from that original assessment pertain to the certified dye.

CHEMISTRY

Definition and Structure
Acid Violet 43 (CAS No. 4430-18-6; technical name benzenesulfonic acid, 2-[(9,10-dihydro-4-hydroxy-9,10-dioxo-1-anthracenyl)amino]-5-methyl-, monosodium salt) is reported to function in cosmetics, according to the International Cosmetic Ingredient Dictionary and Handbook, as a colorant and is classed chemically as an anthraquinone color.3 It conforms to the formula:

Figure 1. Acid Violet 43

Acid Violet 43 is the International Nomenclature of Cosmetic Ingredients (INCI) name for batches of this colorant that have not been certified.3 To identify the certified colorant for labeling purposes in the United States (U.S.), the INCI name Ext. Violet 2 must be used. In the European Union (EU) and in Japan, the INCI names CI 60730 and Murasaki401, respectively, must be used for identification of this colorant.

Physical and Chemical Properties
Acid Violet 43 is a dark violet crystalline powder with a molecular weight of 431.42 (Table 1). It does not absorb in the ultraviolet A (UVA) or UVB range.

Method of Manufacture
Acid Violet 43 can be prepared by sulfonating the anthraquinone color, C.I. 60725 (Figure 2), and converting it to the sodium salt.1
Acid Violet 43, as a non-certified color, is commercially available in different forms with different analytical specifications and impurity profiles. It tends to have more impurities than the certified color, Ext. D&C Violet No. 2, which contains the same color chemically but is of higher purity. The studies summarized in the SCCS Opinion were performed with both Acid Violet 43 and Ext. D&C Violet No. 2. Three batches of each compound were analyzed; the purity of Acid Violet 43, as tested in the studies reported in the SCCS opinion, ranged from 54.4-60.5%, and that of Ext. D&C Violet No. 2 ranged from 84-93.8% (Table 2). The specifications for Ext. D&C Violet No. 2, as described in the conclusion of the CIR safety assessment published in 2001, have not changed. [21CFR74.2602a]

**USE**

**Cosmetic**

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the U.S. 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 FDA’s 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.

Acid Violet 43 is a non-oxidative (i.e., direct) hair dye, but it can also be used in oxidative hair coloring products. Based on 2016 VCRP data, Acid Violet 43 is reported to be used in 48 hair coloring preparations; 23 of the reported uses are in coloring hair rinses. According to the results of the Council survey, in which data were collected in 2015 and 2016, the maximum reported concentrations of use of Acid Violet 43 range from 0.13-0.35%; the highest reported maximum concentration of use is 0.35% in hair tints. In the original safety assessment, Acid Violet 43 was reported to be used in only 2 hair coloring formulations in 1998; at that time, concentration of used data were not available. However, historical FDA data included in that report indicated that, in 1984, Acid Violet 43 was reported to be used in 30 coloring hair formulations at concentrations ≤0.1% and in 1 coloring shampoo at 0.1-1%.

Acid Violet 43 is considered a coal tar hair dye for which regulations require caution statements and instructions regarding patch tests to be used for dyeing the eyelashes or eyebrows; to do so may cause blindness.

Product labels shall also bear patch test instructions for determining whether the product causes skin irritation. The 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. The authors of that report concluded that, in present form, the hair dye self-test has severe limitations. The authors issued the warning 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. An accompanying editorial written 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 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.

In 2013, the SCCS concluded that the use of Acid Violet 43 (as related to batch Ext. D&C Violet No.2 0609RA, purity of 94%) as a non-oxidative hair dye with a maximum on head concentration of 0.5% active dye does not pose a risk to the health of the consumer. The SCCS also stated that a sensitizing potential cannot be excluded. The conclusion of the SCCS opinion stated that “Acid Violet 43 is also used as a colorant, but that use was not assessed in this opinion”.

**Impurities/Composition**

Acid Violet 43, as a non-certified color, is commercially available in different forms with different analytical specifications and impurity profiles. It tends to have more impurities than the certified color, Ext. D&C Violet No. 2, which contains the same color chemically but is of higher purity. The studies summarized in the SCCS Opinion were performed with both Acid Violet 43 and Ext. D&C Violet No. 2. Three batches of each compound were analyzed; the purity of Acid Violet 43, as tested in the studies reported in the SCCS opinion, ranged from 54.4-60.5%, and that of Ext. D&C Violet No. 2 ranged from 84-93.8% (Table 2).

The specifications for Ext. D&C Violet No. 2, as described in the conclusion of the CIR safety assessment published in 2001, have not changed. [21CFR74.2602a]
In the European Union, sodium, 4-[(9,10-dihydro-4-hydroxy-9,10-dioxo-1-anthryl)amino]toluene-3-sulphonate (i.e., Acid Violet 43) is listed in Annex III (i.e., List of Substances Which Cosmetic Products Must Not Contain Except Subject to the Restrictions Laid Down; III/291) of the Cosmetic Regulations (Regulation (EU) No 1190/2015). It is allowed as a hair dye substance in non-oxidative hair dye products at a maximum concentration in a ready-for-use preparation of 0.5%. Sodium 4-[(9,10-dihydro-4-hydroxy-9,10-dioxo-1-anthryl)amino]toluene-3-sulphonate, as CI 60730, is listed in Annex IV (i.e., List of Colorants Allowed in Cosmetics; IV/90) of the Cosmetic Regulations (Regulation (EC) No 1223/2009), with the restriction that it is not to be used in products applied to mucous membranes.

In the United States, Ext. Violet 2 may be safely used for coloring externally applied cosmetics in amounts consistent with good manufacturing practice.[21CFR74.2602a] Externally applied cosmetics may not be used in products that are applied to the lips or any body surface covered by mucous membrane.

**TOXICOKINETIC STUDIES**

**Dermal Penetration**

The percutaneous absorption of Acid Violet 43 (59.3% pure) was evaluated using human skin samples. The skin samples (thickness 380 ± 25 µm) were mounted in diffusion cells and exposed for 30 min to 0.12% Acid Violet 43 (as active dye) in a semi-permanent hair dye formulation (20 mg/cm², corresponding to 25.4 µg active dye/cm²). The skin surface was washed after exposure, and percutaneous absorption was determined 24 h after application by measuring the concentration of Acid Violet 43 in the washing, stratum corneum (isolated via tape stripping), the epidermis + dermis, and the receptor fluid (0.9% saline). Most of the test substance applied on the skin surface was removed by the washing procedure (~104% of the applied dose), and the total calculated recovery was ~105%. No Acid Violet 43 was measured in the receptor fluid. The mean absorbed amount of Acid Violet 43 (estimated as the sum of amounts measured in epidermis, dermis, and receptor fluid, and assuming concentrations at the detection limit in the receptor fluid of 40 ng) was 0.11 ± 0.06 µg active dye/cm² (0.53 ± 0.33% of the applied dose).

Two experiments were performed using porcine ear skin mounted in glass flow-through diffusion chambers to determine the percutaneous absorption of Ext. D&C Violet No. 2 (95% pure). The skin sample thickness was 100-450 µm in Experiment 1 and 800-900 µm in Experiment 2. In the first experiment, 2 mg/ml of a 20% ethanol in a water solution was tested, and in the second experiment, it was 0.5% Ext. D&C Violet No.2 in a semi-permanent hair dye formulation. The per surface area dose of the formulation in both studies was 1000 mg/cm². The amount of test substance placed in the donor chamber was 1 ml in the first experiment and 1.25 ml of the hair dye formulation (corresponding to 5 mg/cm² test substance) in the second experiment. For both studies, the exposure time was 30 min, and the receptor fluid was 20% ethanol in water. The remaining test material was removed at the end of the exposure period, and the donor chambers were filled with 1 ml of receptor fluid; samples were collected from the receptor chamber at several intervals during the exposure period for up to 24 h. Because the stratum corneum was not separated from the epidermal and dermal compartments, the amounts of test material in receptor solution plus that in skin extracts were considered to be absorbed. After 24 h of sampling, the mean total recovery of test material was 88.8 ± 4.08% in Experiment 1 and 90.6 ± 4.67% in Experiment 2. The test substance could not be detected in the receptor fluid at any sampling time or in skin extracts of Experiment 1 (the limit of detection was 500 ng/ml). Maximal flux rates were calculated by assuming the detection limit as the concentration of the test material in the receptor fluid samples. Absorption was estimated by adding skin extract values to the calculated fluxes. Based on the highest values calculated for the flux (19.3 µg or 19.1 µg/cm² in Experiment 1 and 19.0 µg or 24.8 µg/cm² in Experiment 2) and values for skin extracts (1.85 ± 0.01 µg or 1.83 ± 0.01 µg/cm² in Experiment 1, based on the detection limit of 500 ng/ml, and 5.82 ± 2.67 µg or 5.75 ± 2.6 µg/cm² in Experiment 2), the highest rate for skin absorption was estimated as 20.93 µg/cm² (approximately 1%) in Experiment 1 and 30.55 µg/cm² (approximately 0.6%) in Experiment 2.

**Absorption, Distribution, Metabolism, and Excretion**

A toxicokinetic study of Acid Violet 43 (59.8% pure) was performed as part of a bone marrow micronucleus test (described in the Genotoxicity section). In the toxicokinetic study, 3 mice/sex/sampling time were given a single dosage by gavage of 0 or 2000 mg active dye/kg bw (approximately 3348 mg dye/kg bw) in water (20 ml/kg bw). Blood samples were taken 15, 30 or 60 minutes after dosing (single sample per animal). There were no clinical signs of toxicity and no mortality. Oral bioavailability was demonstrated by “high blood levels of Acid Violet 43 observed in all animals (range 5.7-34.6 µg/ml)”.

**TOXICOLOGICAL STUDIES**

**Acute Toxicity Studies**

*The oral LD₅₀ of Ext. D&C Violet No. 2 was >4640 mg/kg for male rats and >2000 mg/kg for mongrel dogs.*
**Short-Term Toxicity Studies**

In a short-term toxicity study using guinea pigs in which dermal applications of a United States Pharmacopeia (USP) hydrophilic ointment containing 0.1% or 1.0% Ext. D&C Violet No. 2 were made 5 days/wk for 3 wks, test article-related gross and microscopic lesions were not observed.1

**Dermal**

In a subchronic toxicity study using rabbits in which dermal applications of a USP hydrophilic ointment containing 0.1% or 1.0% Ext. D&C Violet No. 2 were made 5 days/wk for a total of 65 applications, test article-related gross and microscopic lesions were not observed.1

**Oral**

Groups of 10 male and 10 female Sprague-Dawley rats were administered Acid Violet 43 (54.4% pure) in 5 ml/kg water.2 The doses corresponded to 0, 27, 109 or 435 mg active dye/kg bw/day, respectively. The animals were observed daily, body weights and feed consumption were measured weekly, and hematology, blood clinical chemistry, and urinalysis parameters were determined at study termination. At necropsy, gross and macroscopic examinations were performed, and organ weights, including those of ovaries and testes, were measured. No test article-related mortality was reported. Dose-dependent increases in salivation were observed at all dose levels. Colored urine, feces, hair, and extremities were observed at all doses and were attributed to the staining properties of the test article. No other clinical signs, no ocular findings, and no changes in body weights and feed consumption were reported. Statistically significant changes in blood clinical chemistry parameters were considered to be of no toxicological importance. Several statistically significant changes in hematological parameters were observed; only an increased activated partial thromboplastin time (APTT) in high dose males was considered to be treatment-related. There were no significant differences in organ weights among treated and control groups. Dose-related greenish contents or greenish colorations of the mucosa of the digestive tract were observed at necropsy. No treatment-related microscopic changes were observed. The non-observed adverse effect level (NOAEL) was 200 mg/kg bw/day Acid Violet 43 (109 mg active dye/kg bw/day), based on the increased APTT in the high dose males.

A 13-wk study following the same protocol was performed with Ext. D&C Violet No. 2 (93.8% pure).2 Groups of 10 male and 10 female Wistar rats were dosed daily for 13 wks by gavage with 0, 100, 300, and 1000 mg/kg bw/day Ext. D&C Violet No. 2 (0, 94, 282 or 940 mg active dye/kg bw/day, respectively) in 1% aq. carboxymethylcellulose. None of the animals died during the study. No adverse clinical signs were reported, and there were no changes in body weights or feed consumption. Dark blue feces were observed at all doses and were attributed to staining properties of the test substance. Decreased locomotor activity in high-dose males (observed after 15 min) and in mid- and high-dose females (after 45 min, and persisting in high-dose females) and changes in grip strength were considered unlikely to be test-article related. A statistically significant increase in prothrombin time was reported in low- and high-dose males, and an increase in APTT was reported in high-dose males. Changes observed in blood clinical chemistry laboratory parameters and in urinalysis were considered by the study authors to be of no toxicological significance. Statistically significant changes in some organ weights were considered incidental. According to the researchers, the NOAEL was 1000 mg/kg bw/day Ext. D&C Violet No. 2 (940 mg active dye/kg bw/day). However, the SCCS considered the increased in APTT in high-dose males an adverse effect, and the NOAEL to therefore be 300 mg/kg bw/day Ext. D&C Violet No. 2 (282 mg active dye/kg bw/day).

**Developmental and Reproductive Toxicity Studies**

Groups of 25 female Sprague-Dawley rats were administered Acid Violet 43 (54.4% pure) in water by gavage at a dose of 0, 50, 200 or 800 mg/kg bw/day (equivalent to 0, 27, 109 or 435 mg active dye/kg bw/day) on days 6-15 of gestation.2 The animals were killed on day 20 of gestation. There were 18, 23, 21 and 21 gravid animals in the 0, 50, 200 or 800 mg/kg bw/day groups, respectively. No mortality was reported. Increased salivation was observed in the 800 mg/kg bw/day group. Discolored feces were observed in all groups except the low dose group. Discoloration of placenta was also observed in the high dose group. No effects on litter parameters or fetal weight were reported. According to the researcher, there were no external soft tissue or skeletal anomalies that could be attributed to treatment with the test substance. The NOAEL was 800 mg/kg bw/day (435 mg active dye/kg bw/day) for teratogenicity and for maternal toxicity.

Groups of 22 female Wistar rats were administered 1% Ext. D&C Violet No. 2 (93.8% pure) in carboxymethylcellulose by gavage at doses of 0, 100, 300 and 1000 mg/kg bw/day (equivalent to 0, 94, 282 or 940 mg active dye/kg bw/day) on days 6-17 of gestation.2 The animals were killed on day 21 of gestation. Twenty to 22 females/group were gravid. No mortality was reported. Discolored feces were observed in the high dose group. One low-dose and one high-dose animal had only embryonic resorptions, and one high dose animal had only empty implantation sites; the researcher considered these to be incidental findings (i.e., not likely to be test article-related) due to the lack of an observed dose-dependent response. Fetal and litter incidences of external, soft tissue and skeletal anomalies were similar for control and treated groups. The NOAEL for teratogenicity and maternal toxicity was 1000 mg/kg bw/day (940 mg active dye/kg bw/day).
GENOTOXICITY STUDIES

In Vitro

Ext. D&C Violet No. 2 was not mutagenic in a spot and/or plate test of a Salmonella/mammalian microsome test with or without metabolic activation.†

Acid Violet 43 (54.4% pure) and Ext. D&C Violet No. 2 (95% pure) were not mutagenic in the Ames test when tested at concentrations ≥2720 μg/plate in water or ≥4750 μg active dye/plate in dimethyl sulfoxide (DMSO), respectively (Table 3). In chromosomal aberration assays of Acid Violet 43 (54.4% pure), significant increases in aberrant cell frequency were observed with a 2 h exposure at the highest test concentration (408 μg/ml) with metabolic activation and in the 48-h treatment group at the highest concentration (272 µg/ml) without metabolic activation in human lymphocytes, and structural aberrations were statistically significantly increased in Chinese hamster ovary (CHO) cells at concentrations ≥102 μg active dye/ml. Ext. D&C Violet No. 2, at concentrations ≤342 μg active dye/ml in DMSO, was not genotoxic in a mammalian cell mutation assay in mouse lymphoma cells.

In Vivo

Acid Violet 43 (54.4% pure) was not genotoxic in an unscheduled DNA synthesis assay in which rats were given a single oral dose of up to 816 mg active dye/kg bw in water. Acid Violet 43 (purity 54.4% or 58.8%) was not genotoxic in micronucleus tests in which mice were given a single oral dose of 1088 or 2000 mg active dye/kg bw, respectively, in water.

CARCINOGENICITY

Dermal application of Ext. D&C Violet No. 2 was not carcinogenic to Swiss-Millerton mice.† The majority of the doses (>95) were applied as 1% dispersions in propylene glycol; a total of 103 applications were made.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Sensitization

Animal

The sensitization potential of Acid Violet 43 (60.5% pure) was evaluated in a local lymph node assay (LLNA) performed in accordance with the Organisation for Economic Co-operation and Development (OECD) test guideline 429.2 During induction, 25 μl of the test compound was applied to each ear of female CBA/J mice (4/group) at concentrations of 0.6, 1.5, 3, 6, and 15% active dye (w/v) in acetone/olive oil (AOO, 4/1, v/v; % w/w of ‘pure’ dye based on purity of 60.5%) once daily for 3 days. A vehicle control group and a positive control (25% heptylcinnamaldehyde in AOO) group were treated in the same manner. Lymphocyte proliferation was determined after a 2-day non-treatment period; no lymphoproliferation was observed in any of the animals treated with the test substance. However, a black coloration of the skin of the ears was noted in all treated animals from day 2 up to day 6; this coloration could have concealed possible erythema. The SCCS commented that the highest concentrations of Acid Violet 43 tested (15% of a material that was 60.5% pure – i.e., 9.075%) were too low to exclude a sensitizing potential.

The potential for Ext. D&C Violet No. 2 to induce hypersensitivity was evaluated in a modified guinea pig testing protocol, the adjuvant and patch test.12 As supplied, the commercial-grade test substance contained 990 ppm 1,4-dihydroxy-9,10-anthracenedione (a.k.a. quinizarin); upon purification by precipitation from aqueous solution with ethyl alcohol, after pretreatment of the solution with activated carbon, the 1,4-dihydroxy-9,10-anthracenedione content was <1 ppm. Groups of 14 female Hartley albino guinea pigs were used. Test animals were sensitized with 0.2% 1,4-dihydroxy-9,10-anthracenedione; control groups were not sensitized. One-tenth ml of a water-in-oil emulsion of distilled water:Freund’s complete adjuvant (FCA; 1:1) was injected intradermally into the 4 corners of a 2 cm x 4 cm shaved area of the animals. Grid-like scratches were made at the injection sites, and occlusive patches of 0.1 ml 1% commercial or purified Ext. D&C Violet No. 2 were applied to the injection sites for 24 h. Abrasions and patch application was repeated on the following 2 days. One week after the initial applications, 10% sodium lauryl sulfate in petrolatum was applied to the intradermal injection sites and, on the following day, an occlusive patch of the test material was applied to the same sites for 48 h. After a 2-wk non-treatment period, a test sample was applied to a shaved area of the back of the animals; excess test material was removed after 24 h by washing with acetone. The test sites were scored 1, 24, and 48 h after washing. One animal of the sensitized group had a positive reaction to the commercial-grade substance. None of the animals patch-tested with purified Ext. D&C Violet No. 2 and none of the control animals exposed to either grade of the test material showed any positive reactions.

QSAR

A quantitative structure-activity relationship (QSAR) model was utilized to predict the sensitization potential of all hair dye ingredients registered in Europe (229 substances as of 2004).4 The model predicted Acid Violet 43 to be a strong/moderate sensitizer. The QSAR analysis involved calculating TOPOlogical Substructural MOlecular DESign (TOPS-MODE) descriptors and correlating them to unspecified sensitization data from LLNA that were available in July 2003.
**OCULAR IRRITATION STUDIES**

One-tenth ml of 1% (active dye) Acid Violet 43 (60.5% pure) in water was instilled into the conjunctival sac of the left eye of 3 male New Zealand White rabbits. Slight chemosis was observed in one animal on days 1 and 2, and slight conjunctival erythema was observed in all 3 animals from day 1 up to days 2 or 3. A clear discharge was also noted in one animal on day 2. All reactions were reversible by day 4. It was not known if the “impurities” in the dye contributed to the irritation.

**CLINICAL STUDIES**

**Multicenter Studies**

A total of 736 eczema patients from 3 dermatology clinics were patch-tested with 1% Acid Violet 43 (purity not specified) in petrolatum during a 6-month period in 2007/2008. Twenty mg of the test substance were applied to the upper back of each patient using 8 mm Finn chambers on Scanpor tape. The test sites were scored on days 2, 3, 4, and 7. None of the patients had a positive reaction to the patch test, and there were no irritant reactions. Doubtful reactions were noted in 70 subjects (9.5%); these reactions were all recorded at one test center that considered discoloration of the skin as a doubtful reaction.

**RISK ASSESSMENT**

**Margin of Safety**

The margin of safety (MOS) for the use of Ext. D&C Violet No. 2 (purity 94%) under non-oxidative conditions (maximum on head concentration of 0.5% active dye) was calculated in accordance with OECD test guideline 408, using 50% bioavailability in accordance with the SCCS's Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation. Absorption through the skin (A) = 0.92 µg/cm²

Skin Area surface (SAS) = 580 cm²

Dermal absorption per treatment (SAS x A x 0.001) = 0.534 mg

Typical body weight of human = 60 kg

Systemic exposure dose (SED; SAS x A x 0.001/60) = 0.009 mg/kg bw/d

NOAEL (from 90-day, oral, rat) = 282 mg/kg bw/d

50% bioavailability = 141 mg/kg bw/d

MOS (NOAEL/SED) = 15,667

**EPIDEMIOLOGICAL STUDIES**

Acid Violet 43 is used in non-oxidative hair dyes. 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**

In 2001, the Panel published a safety assessment with the conclusion that Acid Violet 43 is safe for use in hair dye formulations when free of impurities, except for the following: ≤18% volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); ≤0.4% water-insoluble matter; ≤0.2% 1-hydroxy-9,10-anthracenedione; ≤0.2% 1,4-dihydroxy-9,10-anthracenedione; ≤0.1% p-toluidine; ≤0.2% p-toluidine sulfonic acids, sodium salts; ≤1% subsidiary colors, ≤20 ppm lead (as Pb); ≤3 ppm arsenic (as As); ≤1 ppm mercury as (Hg); and ≥80% total color. Acid Violet 43, an anthraquinone color, is a non-certified color; the certified colorant is identified as Ext. Violet 2 (a.k.a., Ext. D&C Violet 2).

Based on 2016 VCRP data, Acid Violet 43 is reported to be used in 48 hair coloring preparations at a maximum reported concentration of use of 0.35% (in hair tints). In the original safety assessment, Acid Violet 43 was reported to be used in only 2 hair coloring formulations in 1998; at that time, concentration of use data were not available. However, according to historical FDA data that were included in that report, in 1984, Acid Violet 43 was reported to be used in 30 coloring hair formulations at concentrations of ≥0.1% and in 1 coloring shampoo at a concentration of 0.1-1%. In Europe, the SCCS concluded that the use of Acid Violet 43 (as related to batch Ext. D&C Violet No. 2, 94% pure) as a non-oxidative hair dye with a maximum on head concentration of 0.5% active dye does not pose a risk to the health of the consumer, but a sensitizing potential could not be excluded.

In a study examining the percutaneous absorption of Acid Violet 43 (59.3% pure) through human skin samples, the mean absorbed amount of Acid Violet 43 (estimated as the sum of amounts measured in epidermis, dermis, and receptor fluid when assuming concentrations at the detection limit in the receptor fluid of 40 ng) was 0.11 ± 0.06 µg active dye/cm² (0.53 ± 0.33% of the applied dose). In studies using porcine ear skin, the highest rates for skin absorption of Ext. D&C Violet No. 2 (95% pure) were estimated as 20.93 µg/cm² (approximately 1%) and 30.55 µg/cm² (approximately 0.6%) in two experiments.
In a toxicokinetic study of Acid Violet 43 (59.8% pure) that was performed as part of a bone marrow micronucleus test, oral bioavailability was demonstrated by high blood levels of Acid Violet 43 observed in all animals.

In a subchronic toxicity study in which Sprague-Dawley rats were dosed by gavage once daily for 13 wks with up to 800 mg/kg bw/day Acid Violet 43 (54.4% pure), the NOAEL was 200 mg/kg bw/day Acid Violet 43 (109 mg active dye/kg bw/day), based on increased APTT in high dose males; no clinical signs of toxicity were reported. The NOAEL in a 13-wk study of Ext. D&C Violet No. 2 (93.8% pure) in rats following the same protocol, with a maximum dose of 1000 mg/kg bw/day, was 300 mg/kg bw/day Ext. D&C Violet No. 2 (282 mg active dye/kg bw/day); again the NOAEL was based on an increased in APTT in high dose males.

Gravid female Sprague-Dawley rats were administered Acid Violet 43 (54.4% pure) in water by gavage at doses up to 800 mg/kg bw/day (i.e., up to 435 mg active dye/kg bw/day) on days 6-15 of gestation. No external soft tissue or skeletal anomalies were attributed to treatment with the test substance, and the NOAEL was 800 mg/kg bw/day for teratogenicity and for maternal toxicity. In a study in which female Wistar rats were administered 1% Ext. D&C Violet No. 2 (93.8% pure) in carboxymethylcellulose by gavage at doses up to 1000 mg/kg bw/day (i.e., up to 940 mg active dye/kg bw/day) on days 6-17 of gestation, the NOAEL for teratogenicity and maternal toxicity was 1000 mg/kg bw/day.

Acid Violet 43 (54.4% pure) and Ext. D&C Violet No. 2 (95% pure) were not mutagenic in the Ames test when tested at concentrations of ≥2720 µg/plate in water or ≥4750 µg active dye/plate in dimethyl sulfoxide (DMSO), respectively. In chromosomal aberration assays of Acid Violet 43 (54.4% pure), significant increases in aberrant cell frequency were observed with a 2-h exposure at the highest test dose (408 g/ml) with metabolic activation and in the 48-h treatment group at the highest concentration (272 µg/ml) without metabolic activation in human lymphocytes, and structural aberrations were statistically significantly increased in CHO cells at concentrations ≥102 µg active dye/ml. Ext. D&C Violet No. 2, at concentrations ≤342 µg active dye/ml in DMSO, was not genotoxic in a mammalian cell mutation assay in mouse lymphoma cells. In vivo studies, Acid Violet 43 (54.4% pure) was not genotoxic in an unscheduled DNA synthesis assay in which rats were given a single oral dose of up to 816 mg active dye/kg bw in water, or in micronucleus tests in which mice were given a single oral dose of 1088 mg active dye/kg bw (54.4% pure) or 2000 mg active dye/kg bw in water.

Concentrations of up to 15% Acid Violet 43 (60.5% pure) active dye (w/v) in AOO did not produce positive results in a mouse LLNA; however, the SCCS commented that the highest concentrations of Acid Violet 43 tested were too low to exclude a sensitizing potential. An adjuvant and patch test for hypersensitivity potential of commercial-grade and purified Ext. D&C Violet No. 2 (containing 990 ppm and <1 ppm 1,4-dihydroxy-9,10-anthracenedione, respectively) was conducted in female Hartley albino guinea pigs that were sensitized with 0.2% 1,4-dihydroxy-9,10-anthracenedione and in control (non-sensitized) guinea pigs. One animal of the sensitized group had a positive reaction to the commercial-grade substance. None of the animals patch-tested with purified Ext. D&C Violet No. 2 and none of the control animals exposed to either grade of the test material showed any positive reactions. A QSAR model predicted Acid Violet 43 to be a strong/moderate sensitizer. Instillation of 1% (active dye) Acid Violet 43 (60.5% pure) into rabbit eyes resulted in slight chemosis in 1/3 animals and slight conjunctival erythema in all animals. All reactions were reversible by day 4.

A total of 736 eczema patients from 3 dermatology clinics were patch-tested with 1% Acid Violet 43 (purity not specified) in petrolatum during a 6-month period. No positive reactions were noted.

The MOS for the use of Ext. D&C Violet No. 2 (purity 94%) under non-oxidative conditions (maximum on head concentration of 0.5% active dye), assuming 50% bioavailability, was calculated as 15,667.

**DISCUSSION**

In 2001, the Panel published a safety assessment of Acid Violet 43 (a non-certified colorant) that utilized safety test data on Ext. D&C Violet No. 2 (a certified color). Acid Violet 43 has the same structure as Ext. D & C Violet No. 2, but because it is not a certified color it may contain impurities at different levels or that are not allowed in the certified color. In the 2001 assessment, the Panel concluded that Acid Violet 43 was safe for use in hair dye formulations when it met the impurity specifications indicated for the certified color.

In accordance with its Procedures, CIR evaluates the conclusions of previously-issued reports every 15 years to determine whether or not the conclusion remains valid. In the time since the original safety assessment was published, test data on Acid Violet 43 have become available; the compounds tested were 54.4-60.5% pure. Because safety data are now available on Acid Violet 43 (the non-certified colorant), and because the results of the studies on Acid Violet 43 were similar to those obtained in studies of Ext. D&C Violet No. 2 (the certified color), the Panel re-opened the report to remove the impurity specifications from the conclusion. The Panel did state that the composition of Acid Violet should be the same or similar to that identified for the test substance that was assessed in this report.
The CIR Expert Panel concluded Acid Violet 43 is safe in the present practices of use and concentration for use in hair dye formulations. (Note: This conclusion supersedes the conclusion published in 2001.)
# TABLES

**Table 1. Physical and chemical properties**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>physical form</td>
<td>crystalline powder</td>
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</tr>
<tr>
<td>Color</td>
<td>dark violet</td>
<td>2</td>
</tr>
<tr>
<td>molecular weight (g/mol)</td>
<td>431.4</td>
<td>2</td>
</tr>
<tr>
<td>water solubility (g/100 ml)</td>
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<td>2</td>
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<tr>
<td>other solubility (g/100 ml)</td>
<td>ethanol: &lt;1</td>
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</tr>
<tr>
<td></td>
<td>dimethyl sulfoxide: &lt;1</td>
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<tr>
<td>log K&lt;sub&gt;ow&lt;/sub&gt;</td>
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<td>2</td>
</tr>
<tr>
<td>UV Absorption (λ) (nm)</td>
<td>λ&lt;sub&gt;max&lt;/sub&gt; 253 nm, 570 nm</td>
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Table 2. Composition of test compounds studied in the SCCS Opinion - Acid Violet 43 and Ext. D&C Violet No. 2

<table>
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<tr>
<th>Units</th>
<th>Acid Violet 43</th>
<th>Ext. D&amp;C Violet No. 2</th>
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<tr>
<td></td>
<td>Sample 1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>Sample 2&lt;sup&gt;1&lt;/sup&gt;</td>
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<tr>
<td>Tacode</td>
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<tr>
<td>Impurities</td>
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<td>Acid Green 25</td>
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<tr>
<td>isomer of Acid Green 25</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>isomer of Acid Violet 43</td>
<td>d</td>
<td>d</td>
</tr>
<tr>
<td>1,4-dihydroxyanthraquinone</td>
<td>%</td>
<td>&lt;0.25</td>
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<tr>
<td>1-hydroxy-9,10-anthracenedione</td>
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<tr>
<td>p-toluidine</td>
<td>%</td>
<td>-</td>
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<tr>
<td>p-toluidine sulfonic acids, sodium salts</td>
<td>%</td>
<td>-</td>
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<tr>
<td>Subsidiary colors</td>
<td>%</td>
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<td>Heavy metals (ICP-OES)</td>
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</tr>
<tr>
<td>Fe</td>
<td>mg/kg</td>
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<tr>
<td>Al</td>
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<td>Ag, As, Ba, Bi, Cd, Co, Cr, Mo, Ni, Pb, Pt, Sb, Se, Ti, V, Zn</td>
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<tr>
<td>Hg</td>
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<td>Ash Content</td>
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<td>Water content (K.F. method)</td>
<td>g/100 g</td>
<td>9.3</td>
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<td>Starch content (expressed as glucose; HPIC)</td>
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<td>Sulfate ions (expressed as Na&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;; HPIC)</td>
<td>g/100 g</td>
<td>0.43</td>
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<tr>
<td>Chloride ions (expressed as NaCl; potentiometry)</td>
<td>g/100 g</td>
<td>4.2</td>
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<tr>
<td>sum of volatile matter</td>
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<tr>
<td>water-insoluble matter</td>
<td>%</td>
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<tr>
<td>residual solvents (GC)</td>
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<td>n.d.</td>
</tr>
<tr>
<td>mixed oxides</td>
<td>%</td>
<td>-</td>
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</tbody>
</table>

<sup>1</sup>chemical characterization performed by nuclear magnetic resonance, infrared spectroscopy, and mass spectrometry

<sup>2</sup>spectrophotometric determination, at 570 nm

d - detected but not quantified due to lack of reference standard

n.d. – not detected

"-" - no data

* - sodium chloride + sodium sulfate 8%
Table 3. Genotoxicity studies

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Purity</th>
<th>Concentration/Vehicle</th>
<th>Test System</th>
<th>Procedure</th>
<th>Results</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td><strong>IN VITRO</strong></td>
<td></td>
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</tr>
<tr>
<td>Acid Violet 43</td>
<td>54.4%</td>
<td>170, 340, 680, 1360, and 2720 µg/plate; in water</td>
<td><em>Salmonella typhimurium</em> TA1535, TA1537, TA98, and TA100 and in <em>Escherichia coli</em> WP2uvrA</td>
<td>Ames test, with and without metabolic activation; vehicle and valid positive controls were used</td>
<td>negative</td>
<td>2</td>
</tr>
<tr>
<td>Ext. D&amp;C Violet No. 2</td>
<td>95%</td>
<td>31.35, 95, 316.35, 950, 2375, 4750 μg active dye/plate in DMSO</td>
<td><em>S. typhimurium</em> TA1535, TA1537, TA98, and TA100 and in <em>E. coli</em> WP2uvrA</td>
<td>OECD 471; Ames test with and without metabolic activation; vehicle and valid positive controls were used</td>
<td>negative</td>
<td>2</td>
</tr>
<tr>
<td>Acid Violet 43</td>
<td>54.4%</td>
<td>68, 136 and 272 µg active dye/ml without metabolic activation 85, 170 and 340 µg active dye/ml with metabolic activation; vehicle not given 68, 204 and 272 µg active dye/ml without metabolic activation 68, 272 and 408 µg active dye/ml with metabolic activation</td>
<td>human lymphocytes</td>
<td>OECD 473; chromosomal aberration assay; 2-h exposure in the presence and 24-, and 48 h exposures in the absence of metabolic activation; negative and valid positive controls were used 2-h exposure in the presence and 24-h exposure in the absence of metabolic activation; negative and valid positive controls were used</td>
<td>24 h harvest – negative 48 h harvest - significant increase in aberrant cell frequency with the 2 h exposure at the highest test dose (408 µg/ml) with metabolic activation and in the 48-h treatment group at the highest concentration (272 µg/ml) without metabolic activation</td>
<td>2</td>
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<tr>
<td>Acid Violet 43</td>
<td>54.4%</td>
<td>272, 544 and 2720 µg active dye/ml, with and without metabolic activation in distilled water 102, 204 and 408 µg active dye/ml (20 h harvest time) and 51, 102 and 204 µg active dye/ml (44 h harvest time) without metabolic activation 680, 1360 and 2720 µg active dye/ml with metabolic activation 20 and 44 h harvest times)</td>
<td>CHO cells</td>
<td>OECD 473; chromosomal aberration assay; 3- to 4-h exposure in the presence of metabolic activation; vehicle and valid positive controls were used 3- to 4-h exposure in the presence of metabolic activation; continuous exposure in the absence of metabolic activation; vehicle and valid positive controls were used</td>
<td>structural aberrations were statistically significantly increased in both experiments and at both harvest times at concentrations ≥102 µg active dye/ml in the presence and absence of activation; some of these increases were observed in the absence of overt cytotoxicity</td>
<td></td>
</tr>
<tr>
<td>Ext. D&amp;C Violet No. 2</td>
<td>95%</td>
<td>5.3, 10.7, 21.4, 42.8, 85.5 and 171 µg active dye/ml in DMSO 21.4, 42.8, 85.5, 171 and 342 µg active dye/ml in DMSO</td>
<td>mouse lymphoma cell line L5178Y/TK−/−</td>
<td>OECD 476; mammalian cell gene mutation assay; 4 h treatment time; with and without metabolic activation; vehicle and valid positive controls were used</td>
<td>negative</td>
<td></td>
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<tr>
<td><strong>IN VIVO</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Acid Violet 43</td>
<td>54.4%</td>
<td>0, 82 and 816 mg active dye/kg bw in water</td>
<td>3 male Wistar rats/group</td>
<td>OECD 482; unscheduled DNA synthesis; animals were given a single oral dose; animals of the vehicle control and low dose group were killed 16 h after dosing; animals given the high dose were killed 2 or 16 h after dosing; 2-AAF was used as a positive control</td>
<td>negative</td>
<td>2</td>
</tr>
<tr>
<td>Acid Violet 43</td>
<td>54.4%</td>
<td>0 and 1088 mg active dye/kg bw in water</td>
<td>5 Swiss mice/sex/group</td>
<td>OECD 474; micronucleus test; each animal was given a single dose by gavage and killed 24 or 48 h after dosing; CPA was used as a positive control</td>
<td>negative</td>
<td>2</td>
</tr>
<tr>
<td>Acid Violet 43</td>
<td>59.8%</td>
<td>0 and 2000 mg active dye/kg bw in purified water</td>
<td>5 Swiss mice/sex/group</td>
<td>OECD 474; micronucleus test; each animal was given a single dose by gavage and killed 24 or 48 h after dosing; CPA was used as a positive control</td>
<td>negative</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: 2-AAF - 2-acetylaminofluorene; CHO – Chinese hamster ovary; CPA - cyclophosphamide; DMSO – dimethyl sulfoxide; OECD – Organisation for Economic Cooperation and Development
REFERENCES


Final Report on the Safety Assessment of Acid Violet 43

Acid Violet 43 is an anthraquinone color that may be used as a colorant in cosmetic formulations that are hair dyes, colors, and coloring rinses. Batches of Acid Violet 43 that are certified to meet the United States Food and Drug Administration (U.S. FDA) specifications are termed Ext. D & C Violet No. 2. Hair dyes and colors containing Acid Violet 43 are considered coal tar ingredients and, as such, routinely bear a caution statement regarding potential skin irritation and instructions for determining whether the product causes skin irritation in any given individual. Expected concentrations of use are less than or equal to 1%. Impurities include anthracenedione derivatives, p-toluidine, and p-toluidine sulfonic acid, as well as heavy metals. Based on extensive safety test data, the U.S. FDA has established specifications (including limits on impurities) for Ext. D & C Violet No. 2 that allow its use in any cosmetic. It is the certified color (Ext. D & C Violet No. 2) that has been evaluated in the following safety tests. Oral toxicity tests do not demonstrate significant acute toxicity. In a short-term dermal toxicity study using guinea pigs and a subchronic dermal toxicity study using rabbits, no signs of systemic toxicity and no significant local skin reactions were noted. This ingredient was not genotoxic in bacterial assays, nor was it carcinogenic when applied to mouse skin at a 1% concentration. Accordingly, Acid Violet 43 was determined to be safe for use in hair dye formulations, when impurities are limited as follows: ≤18% volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); ≤0.4% water-insoluble matter; ≤0.2% 1-hydroxy-9,10-anthracenedione; ≤0.2% 1,4-dihydroxy-9,10-anthracenedione; ≤0.1% p-toluidine; ≤0.2% p-toluidine sulfonic acids, sodium salts; ≤1% subsidiary colors; ≤20 ppm lead (as Pb); ≤3 ppm arsenic (as As); ≤1 ppm mercury (as Hg); and with ≥80% total color.

INTRODUCTION

Acid Violet 43 is an anthraquinone color that functions as a colorant in cosmetic formulations (Wenninger, Canterbery, and McEwen 2000). Batches of Acid Violet 43 that are certified by the Food and Drug Administration (FDA) as meeting the standards and specifications described in the Code of Federal Regulations (21 CFR 73 & 74) are named Ext. D & C Violet No. 2 (FDA 1976). Most of the available safety test data are from studies of Ext. D & C Violet No. 2 conducted to support FDA listing this color for drug and cosmetic use.

CHEMISTRY

Definition and Structure

Acid Violet 43 (CAS No. 4430-18-6) is an anthraquinone color that conforms to the formula shown in Figure 1 (Wenninger, Canterbery, and McEwen 2000).

Acid Violet 43 and Ext. D & C Violet No. 2 are also known as CI 60730: 2-[(9,10-Dihydro-4-Hydroxy-9,10-Dioxo-1-Anthracenyl)Amino]-5-Methylbenzenesulfonic Acid, Monosodium Salt; Benzenesulfonic Acid, 2-[(9,10-Dihydro-4-Hydroxy-9,10-Dioxo-1-Anthracenyl)Amino]-5-Methyl-, Monosodium Salt (Wenninger, Canterbery, and McEwen 2000); Monosodium Salt of 1,5-Bis(o-Sulfo-p-Toluuido) Anthraquinone (Hazelton Laboratories, Inc. 1968); Monosodium Salt of 1-Hydroxy-4-(Sulfo-p-Toluuido)-Anthraquinone (American Cyanamid Company 1965; 1966; 1967); and 1(2-Sulfo-p-Toluidino)4-Hydroxy-Anthraquinone (Brown and Brown 1976).

Physical and Chemical Properties

Acid Violet 43 has a bluish violet hue in daylight and is slightly redder in artificial light (tungsten) (Society of Dyers and Colourists 1971a). It is soluble in alcohol (Society of Dyers and Colourists 1971b) and water (Hazelton Laboratories, Inc. 1968).

Manufacture and Production

Acid Violet 43 can be prepared by sulfonating the anthraquinone color, C.I. 60725, shown in Figure 2 and converting it to the sodium salt (Society of Dyers and Colourists 1971b). In 1980, U.S. production of Acid Violet 43 was >4.5 x 10^5 kg (Sigman et al. 1985).

Analytical Methods

Acid Violet 43 has been determined using ion-pair reverse-phase high-performance liquid chromatography with ultraviolet-visible (UV-vis) detection (Gagliardi et al. 1987; Wegener et al. 1987).

Ultraviolet Absorbance

Published data on the ultraviolet absorbance of Acid Violet 43 were not found.
Impurities

Ext. D & C Violet No. 2 must be free of impurities except for the following:  ≤18% volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); ≤0.4% water-insoluble matter; ≤0.2% 1-hydroxy-9,10-anthracenedione; ≤0.2% 1,4-dihydroxy-9,10-anthracenedione; ≤0.1% p-toluidine; ≤0.2% p-toluidine sulfonic acids, sodium salts; ≤1% subsidiary colors; ≤20 ppm lead (as Pb); ≤3 ppm arsenic (as As); ≤1 ppm mercury (as Hg); and ≥80% total color (FDA 1976).

Under normal conditions of use, skin contact with hair dye is restricted to 30 minutes.

U.S. laws and regulations prohibit any cosmetic product intended for sale in the U.S. from containing a colorant that has not been previously approved by the FDA and that does not meet applicable standards and specifications as found in 21 CFR 73 & 74 (Wenninger, Canterbery, and McEwen 2000). Each batch of approved synthetic organic colorant must be certified by the FDA as meeting these standards and specifications. An exception exists for coal tar colorants used in hair dye products, such as Acid Violet 43, which also meet other specific requirements. Accordingly, the reported uses of Acid Violet 43 in hair dyes and colors and coloring rinses are allowable under the Food, Drug, and Cosmetic Act, but the use of Acid Violet 43 in deodorants is not; for that use, only Ext. D & C Violet No. 2 is permitted.

hair coloring formulations are applied to or can come in contact with hair, skin (particularly at the scalp), eyes, and nails. Individuals dyeing their hair could use such formulations once every few weeks, whereas hairdressers could come in contact with products containing these ingredients several times a day.

### TABLE 1

<table>
<thead>
<tr>
<th>Product category</th>
<th>Total no. of formulations in category</th>
<th>Total no. containing ingredient</th>
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<td>Hair dyes and colors</td>
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<tr>
<td>Hair rinses (coloring)</td>
<td>32</td>
<td>1</td>
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<tr>
<td>Deodorants (underarm)*</td>
<td>241</td>
<td>1</td>
</tr>
<tr>
<td><strong>1998 total</strong></td>
<td><strong>3</strong></td>
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</table>

*According to the FDA, use of a noncertified color in cosmetic formulations other than hair dyes having caution statements and patch test instructions is a violation of the Food, Drug, and Cosmetic Act.

### TABLE 2

<table>
<thead>
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<th>Product category</th>
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<th>0%–0.1%</th>
<th>Total</th>
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</thead>
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<tr>
<td>Hair dyes/colors (requiring caution statements)</td>
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<td>25</td>
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<tr>
<td>Hair shampoos (coloring)</td>
<td>1</td>
<td>4</td>
<td>5</td>
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<tr>
<td>Hair bleaches</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1</strong></td>
<td><strong>30</strong></td>
<td><strong>31</strong></td>
</tr>
</tbody>
</table>

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**Caution**—This product contains ingredients that may cause skin irritation on certain individuals, and a preliminary test according to accompanying directions should be made. This product must not be used for dyeing eyelashes or eyebrows; to do so may cause blindness.
At its February 11, 1992, meeting, the Cosmetic Ingredient Review (CIR) Expert Panel issued the following policy statement on coal tar hair dye product labeling:

The Cosmetic Ingredient Review (CIR) Expert Panel has reviewed the cosmetic industry's current coal tar hair dye product labeling, which recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 24 hours after application of the test material and prior to the use of a hair dye formulation. Since the recommendation on the industry's adopted labeling establishes a procedure for individual user safety testing, it is most important that the recommended procedure be consistent with current medical practice.

There is a general consensus among dermatologists that screening patients for sensitization (allergic contact dermatitis) should be conducted by the procedures used by the North American Contact Dermatitis Group and the International Contact Dermatitis Group (North American Contact Dermatitis Group, 1980; Eiermann et al., 1982; Adams et al., 1985). These procedures state that the test material should be applied at an acceptable concentration to the patient, covered with an appropriate occlusive patch, and evaluated for sensitization 48 and 72 hours after application. The CIR Expert Panel has cited the results of studies conducted by both the North American Contact Dermatitis Group and the International Contact Dermatitis Group in its safety evaluation reports on cosmetic ingredients (Elder, 1985). During the August 26-27, 1991, public meeting of the CIR Expert Panel, all members agreed that the cosmetic industry should change its recommendation for the evaluation of the open patch test from 24 hours to 48 hours after application of the test material.

The industry was advised of this recommendation and asked to provide any compelling reasons why this recommendation should not be made by the Expert Panel and adopted by the cosmetic industry. No opposition to this recommendation was received. At the February 11, 1992, public meeting of the CIR Expert Panel, this policy statement was adopted.

**International**

According to the European Community Directive, Acid Violet 43 (as CI 60730) is a coloring agent allowed exclusively in cosmetic products intended not to come into contact with mucous membranes (Cosmetics Directive of the European Union 1995).

Acid Violet 43, as Violet No. 401, is listed in the Japanese Comprehensive Licensing Standards of Cosmetics by Category (CLS) and has precedent for use without restriction in all CLS categories except eyeliner, lip, and oral preparations. The aluminum lake of this coal-tar colorant is not to be used in cosmetic formulations (Santucci 1999). According to Notification 990 of the Pharmaceutical and Medical Safety Bureau of the Japan Ministry of Health and Welfare, issued September 29, 2000. Acid Violet 43 is not prohibited or restricted in its use beyond a basic obligation of manufacturers to use all ingredients in a manner that guarantees safety (Japan Ministry of Health and Welfare 2000).

**GENERAL BIOLOGY**

Published data on either Acid Violet 43 or Ext. D & C Violet No. 2, which would normally be summarized in this General Biology section, including absorption, distribution, metabolism, and excretion data, were not found.

**ANIMAL TOXICOLOGY**

**Acute Oral Toxicity**

Groups of five male albino rats were given a single oral dose of 100, 215, 464, 1000, 2150, or 4640 mg/kg Ext. D & C Violet No. 2, 84% pure, as a 10% or 25% w/v suspension in a 0.5% aqueous methylcellulose solution (Hazleton Laboratories, Inc. 1962). The animals were killed for necropsy after 7 days. All animals survived until study termination. Coloration of the feces was observed for animals of the two highest dose groups at 24 hours and coloration of the urine was observed for animals of the highest dose group at 4 hours. All, three, and one animal(s) of the 464-, 2150-, and 4640-mg/kg dose groups, respectively, had granular-appearing spleens, and three animals of the 4640-mg/kg dose group had renal congestion. The oral LD₅₀ of Ext. D & C Violet No. 2 was >4640 mg/kg for male albino rats.

Groups of one male and one female adult mongrel dogs were given a single oral dose, by capsule, of 500, 1000, 1500, or 2000 mg/kg of Ext. D & C Violet No. 2 (Hazleton Laboratories, Inc. 1963). All animals were killed for necropsy after 7 days. All animals survived until study termination. With the exception of some staining of the feces, no significant observation were noted. The oral LD₅₀ of (Acid Violet 43) was >2000 mg/kg for mongrel dogs.

**Short-Term Dermal Toxicity**

A United States Pharmacopeia (USP) hydrophilic ointment containing 0.1% or 1.0% Ext. D & C Violet No. 2 was applied to an abraded area of the back of five male albino guinea pigs 5 days per week for 3 weeks for a total of 15 applications (American Cyanamid Company 1966). A control group of 10 guinea pigs was dosed with the ointment without (Acid Violet 43). Five hundred mg/kg of the ointment was applied, the test site was not covered, and the animals were not restrained. The animals were observed daily for signs of toxicity and dermal irritation, and body weights were determined weekly. The animals were killed and necropsied after 3 weeks of dosing. None of the animals died during the study. Signs of toxicity were not noted and significant skin irritation was not observed. As the study progressed, the test site became discolored and it was difficult to determine whether erythema was present; however, edema was not observed. Significant differences were not observed in mean body weights, mean or relative liver weights, or mean or relative kidney weights between test and control animals. Test article–related gross or microscopic lesions were not observed.

**Subchronic Dermal Toxicity**

A USP hydrophilic ointment containing 0.1% or 1.0% Ext. D & C Violet No. 2 was applied to intact skin of the back of five male albino rabbits 5 days per week for a total of 65 applications
(American Cyanamid Company 1965). A control group of 10 rabbits was dosed with the ointment without Ext. D & C Violet No. 2. Five hundred mg/kg of the ointment was applied, the test sites were not covered, and the animals were not restrained. The animals were observed daily for signs of toxicity and dermal irritation. Body weights were determined weekly. The animals were killed and necropsied at study termination.

None of the animals died during the study. Differences in signs of toxicity were not noted between the test and control animals. Some animals had nasal or ocular discharge, and their skin “occasionally appeared dry and thickened.” The skin at the test site appeared slightly pink in animals of the control group and was discolored violet in animals of the test groups, but erythema and edema were not observed. Significant differences were not observed in mean body weights or mean or relative kidney weights between test and control animals. Mean liver weights were statistically significantly decreased for animals of the test groups as compared to the controls, but a significant difference in absolute liver weights was not observed; the researchers concluded that these differences “could be coincidental and unrelated to applications of the dye.” Test article-related gross or microscopic lesions were not observed.

**Dermal Sensitization**

Published data on the sensitization potential of either Acid Violet 43 or Ext. D & C Violet No. 2 using animals were not found.

**Ocular Irritation**

Published data on the ocular irritation potential of either Acid Violet 43 or Ext. D & C Violet No. 2 were not found.

**REPRODUCTIVE AND DEVELOPMENTAL TOXICITY**

Published data on the reproductive and developmental toxicity of either Acid Violet 43 or Ext. D & C Violet No. 2 were not found.

**GENOTOXICITY**

The mutagenic potential of Ext. D & C Violet No. 2 was examined in spot and/or plate tests using *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538, TA1978, and TA98 with and without metabolic activation (Brown and Brown 1976). Negative and positive controls were used. Ext. D & C Violet No. 2 was not mutagenic.

The mutagenic potential of Ext. D & C Violet No. 2 was determined in a *Salmonella/mammalian microsome test* using *S. typhimurium* strains TA98, TA1537, TA100, and TA1535 with and without metabolic activation (Muzzall and Cook 1979). Ext. D & C Violet No. 2 was not mutagenic.

**CARCINOGENICITY**

One hundred female Swiss-Millerton mice, grouped 10 per cage, received weekly dermal applications of Ext. D & C Violet No. 2 to a shaved scapular area of the back (American Cyanamid Company 1967). A control group of 200 mice received weekly applications of vehicle. The test animals were entered into the study over a period of 6 weeks. For the first dose, 0.1 ml of a 1% aqueous solution of Ext. D & C Violet No. 2 was applied with a syringe; this method of dosing was unsatisfactory because the solution did not wet the skin of the animals. Doses 2 through 6 were applied with a “camel’s hair” brush as 2% dispersions in propylene glycol, and doses 7 through 103 were applied as 1% dispersions in propylene glycol. The applications were made over a 107-week period. The test and control animals were killed when test group survival approached 30% of the original 10 animals of the group. The animals were necropsied over weeks 102 to 107. Observations were made daily for dead animals and, after dosing, for presence of papillomas or other neoplasms. Body weights were determined at 6-month intervals. Microscopic examination was performed on tissues of 10 animals of the test and control groups; animals with the “greatest array of grossly evident lesions” were chosen for microscopic examination. Neoplasms from other animals, with the exception of some “obvious leukemias,” were also examined microscopically.

It was estimated that the test animals received approximately 27 mg/kg of Ext. D & C Violet No. 2 each week. Survival of test animals was similar to or slightly greater than that of controls. The probability of occurrences of neoplasms or of leukemia in the test mice was no different than, or significantly lower than, that of controls. Ext. D & C Violet No. 2-induced gross or microscopic lesions were not observed.

**CLINICAL ASSESSMENT OF SAFETY**

**Irritation and Sensitization**

Published data on the clinical irritation and sensitization potential of either Ext. D & C Violet No. 2 or Acid Violet 43 were not found.

**Epidemiology**

Between 35% and 45% of American women dye their hair, often at monthly intervals, over a period of years. This estimate is drawn from market research data on hair dye product use, generally from females aged 15 to 60 (Cosmetic, Toiletry, and Fragrance Association 1993).

A number of epidemiologic studies have investigated the association between cancer and occupation as a hairdresser or barber, or between cancer and personal use of hair dyes. The World Health Organization’s International Agency for Research on Cancer (IARC) empaneled a Working Group on the Evaluation of Carcinogenic Risks to Humans to review all available data on these issues. The Working Group met October 6-13, 1992, in Lyon, France (IARC 1993).

The charge to the IARC Working Group was to ascertain that all appropriate data had been collected and were being reviewed, to evaluate the results of the epidemiological and experimental studies and prepare accurate summaries of the data, and to make
an overall evaluation of the carcinogenicity of the exposure to humans.

The IARC Working Group concluded that "There is inadequate evidence that personal use of hair colourants entails exposures that are carcinogenic." Hence, "Personal use of hair colourants cannot be evaluated as to its carcinogenicity (Group 3)." The IARC Working Group also concluded that "There is limited evidence that occupation as a hairdresser or barber entails exposures that are carcinogenic." Hence, "Occupation as a hairdresser or barber entails exposures that are probably carcinogenic (Group 2A)" (IARC 1993). The Expert Panel concludes that the relevance of the occupational data and conclusion to individuals using hair dyes is unclear.

SUMMARY

Acid Violet 43 is a hair colorant. In order to use this colorant in cosmetic products other than hair dyes and colors, it must be certified by the FDA. Certified batches of Acid Violet 43 must meet specifications described in 21 CFR 73 & 74 and are designated Ext. D & C Violet No. 2. In 1998, frequency of use data submitted to the FDA reported that Acid Violet 43 was used in three formulations, including an underarm deodorant. The use of Acid Violet 43 in an underarm deodorant is in violation of the Food, Drug, and Cosmetic Act. In 1984, it was reported to the FDA that Acid Violet 43 was used at concentrations ≤1%.

Each batch of approved synthetic organic colorant must be certified by the FDA as meeting applicable standards and specifications found in 21 CFR 73 & 74. Ext. D & C Violet No. 2 must be free of impurities except for the following: ≤18% volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); ≤0.4% water-insoluble matter; ≤0.2% 1-hydroxy-9,10-anthracenedione; ≤0.2% 1,4-dihydroxy-9,10-anthracenedione; ≤0.1% p-toluidine; ≤0.2% p-toluidine sulfonic acids, sodium salts; ≤1% subsidiary colors; ≤20 ppm lead (as Pb); ≤3 ppm arsenic (as As); ≤1 ppm mercury (as Hg); and ≥80% total color.

However, coal tar hair dye products, which meet other specifications, do not have to meet these standards and specifications. The hair dyes containing Acid Violet 43, as coal tar hair dye products, are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The following caution statement should be displayed conspicuously on the labels of coal tar hair dyes:

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

The oral LD₅₀ of Ext. D & C Violet No. 2 was >4640 mg/kg for male rats and >2000 mg/kg for mongrel dogs. In a short-term toxicity study using guinea pigs and a subchronic toxicity study using rabbits, in which dermal applications of a USP hydrophilic ointment containing 0.1% or 1.0% Ext. D & C Violet No. 2 were made, test article-related gross and microscopic lesions were not observed.

Ext. D & C Violet No. 2 was not mutagenic in a spot and/or plate test of a Salmonella/mammalian microsome test with or without metabolic activation. Ext. D & C Violet No. 2 was not carcinogenic upon dermal application to mice.

DISCUSSION

The Expert Panel determined that the data provided in this report are sufficient to assess the safety of the tested ingredient as a hair dye ingredient. The Expert Panel recognized that the ingredient tested in available safety studies was Ext. D & C Violet No. 2, which is a certified dye. Acid Violet 43 has the same structure as Ext. D & C Violet No. 2, but it is not a certified color and it could have impurities that are not allowed in the certified color. During the open public discussion of this report at the March 20, 1998, meeting of the CIR Expert Panel, it was reported that, for example, one reason a dye batch of Ext. D & C Violet No. 2 would be rejected for certification is elevated amounts of p-toluidine. Such elevated amounts would be a concern.

The CIR Expert Panel concluded that Acid Violet 43 could be used safely in hair colorants if the impurities were minimized. Therefore, the Expert Panel determined that Acid Violet 43 is safe for use in hair dye formulations if it conforms to the following specifications: ≤18% volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); ≤0.4% water-insoluble matter; ≤0.2% 1-hydroxy-9,10-anthracenedione; ≤0.2% 1,4-dihydroxy-9,10-anthracenedione; ≤0.1% p-toluidine; ≤0.2% p-toluidine sulfonic acids, sodium salts; ≤1% subsidiary colors; ≤20 ppm lead (as Pb); ≤3 ppm arsenic (as As); ≤1 ppm mercury (as Hg); and ≥80% total color.

The Expert Panel noted and was concerned with the similarity of Acid Violet 43 to the dermal carcinogen, 2-anthramine. However, a dermal carcinogenicity study summarized in this review was negative.

The Expert Panel recognizes that irritation and sensitization data on Acid Violet 43 were not available. These data were not requested because hair dyes containing Acid Violet 43, as coal tar hair dye products, are exempt from the principal adulteration provision and from the color additive provisions in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when the label bears a caution statement and patch test instructions for determining whether the product causes skin irritation. The Expert Panel expects that following this procedure will identify prospective individuals who would have an irri-

CONCLUSION

The CIR Expert Panel concludes that Acid Violet 43 is safe for use in hair dye formulations when free of impurities except for the following: ≤18% volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); ≤0.4% water-insoluble
COSMETIC INGREDIENT REVIEW

matter; ≤0.2% 1-hydroxy-9,10-anthracenedione; ≤0.2% 1,4-dihydroxy-9,10-anthracenedione; ≤0.1% p-toluidine; ≤0.2% p-toluidine sulfonic acids, sodium salts; ≤1% subsidiary colors; ≤20 ppm lead (as Pb); ≤3 ppm arsenic (as As); ≤1 ppm mercury (as Hg); and ≥80% total color.

REFERENCES


Cosmetic, Toiletry, and Fragrance Association (CTFA). 1993. Unpublished data on product usage provided by CTFA.


Hazleton Laboratories, Inc. 1968. Petition to FDA dated July 1 to list Ext. D & C Violet No. 2 as suitable and safe for use in externally applied cosmetics. Unpublished data contained in CTFA’s FDA Masterfile. (20 pages.)


Washington, DC: CTFA.


England: Society of Dyers and Colourists with acknowledgment to the American Association of Textile Chemists and Colourists.


England: Society of Dyers and Colourists with acknowledgment to the American Association of Textile Chemists and Colourists.


Washington, DC: CTFA.

Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, N.W., suite 310, Washington, DC 20036, USA.
<table>
<thead>
<tr>
<th>Category</th>
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Memorandum

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

FROM: Beth A. Lange, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: June 24, 2016

SUBJECT: Comments on the Tentative Report: Safety Assessment of Acid Violet 43 as Used in Cosmetics (released June 14, 2016)

Cosmetic Use - As the CIR report states how CI 60730 can be used in cosmetic products in Europe, it would be helpful to state how Ext. Violet 2 can be used in cosmetic products in the United States. Because it is an external color, Ext. Violet 2 cannot be used in products applied to the lips or to any body surface covered by mucous membranes.

Sensitization - The statement that “The SCCS commented that the highest concentrations of Acid Violet 43 tested (60.5% pure) were too low to excluded a sensitizing potential.” is misleading as it suggests 60.5% was the highest concentration tested. The Summary states it correctly, the maximum concentration tested was 15% of a material that was 60.5% pure (or 9.075%).

Margin of Safety, Summary - Although not specifically stated in the SCCS Opinion, the margin of safety calculation assumed use at 0.5% in a direct hair dye product. This should be stated in the CIR report.

Table 3 - In the results column of the first Acid Violet 43 study, the units with 408 need to be corrected from “g/ml” to “μg/ml”