
Amended Safety Assessment of Acid Violet 43 as Used in Cosmetics

Status: Re-Review for Panel Review
Release Date: May 13, 2016
Panel Meeting Date: June 6-7, 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/Writer.

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Monice M. Fiume *MMF*
Assistant Director/Senior Scientific Analyst
Date: May 13, 2016
Subject: Amended Safety Assessment of Acid Violet 43 as Used in Cosmetics

Enclosed is the Re-Review of the Amended Safety Assessment of Acid Violet 43 as Used in Cosmetics (*acidvi062016rep*). In 2001, the Panel published a safety assessment (*acidvi062016prev*) with the conclusion that Acid Violet 43 is safe for use in hair dye formulations when free of impurities except for the following: $\leq 18\%$ volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); $\leq 0.4\%$ water-insoluble matter; $\leq 0.2\%$ 1-hydroxy-9,10-anthracenedione; $\leq 0.2\%$ 1,4-dihydroxy-9,10-anthracenedione; $\leq 0.1\%$ *p*-toluidine; $\leq 0.2\%$ *p*-toluidine sulfonic acids, sodium salts; $\leq 1\%$ subsidiary colors; ≤ 20 ppm lead (as Pb); ≤ 3 ppm arsenic (as As); ≤ 1 ppm mercury as (Hg); and $\geq 80\%$ total color. These impurities 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. 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.

Because it has been 15 years since the original safety assessment was published, the Panel is being asked to determine whether the conclusion should be reaffirmed, or if a re-review is warranted.

Concentration of use data were received from the Council (*acidvi062016data*). Also included are 2016 FDA VCRP data (*acidvi062016FDA*). Acid Violet 43 is reported to be used in 48 hair coloring preparations at a maximum reported concentration of use of 0.35%. 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, 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 $\leq 0.1\%$ and in 1 coloring shampoo at a concentration of 0.1-1%.

The European Commission Scientific Committee on Consumer Safety issued an Opinion on Acid Violet 43 in 2013. 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; the SCCS stated that Acid Violet 43 was not assessed in the opinion. The Opinion summarized test data on both Acid Violet 43 and Ext. D&C Violet No. 2; that information is included in this re-review document.

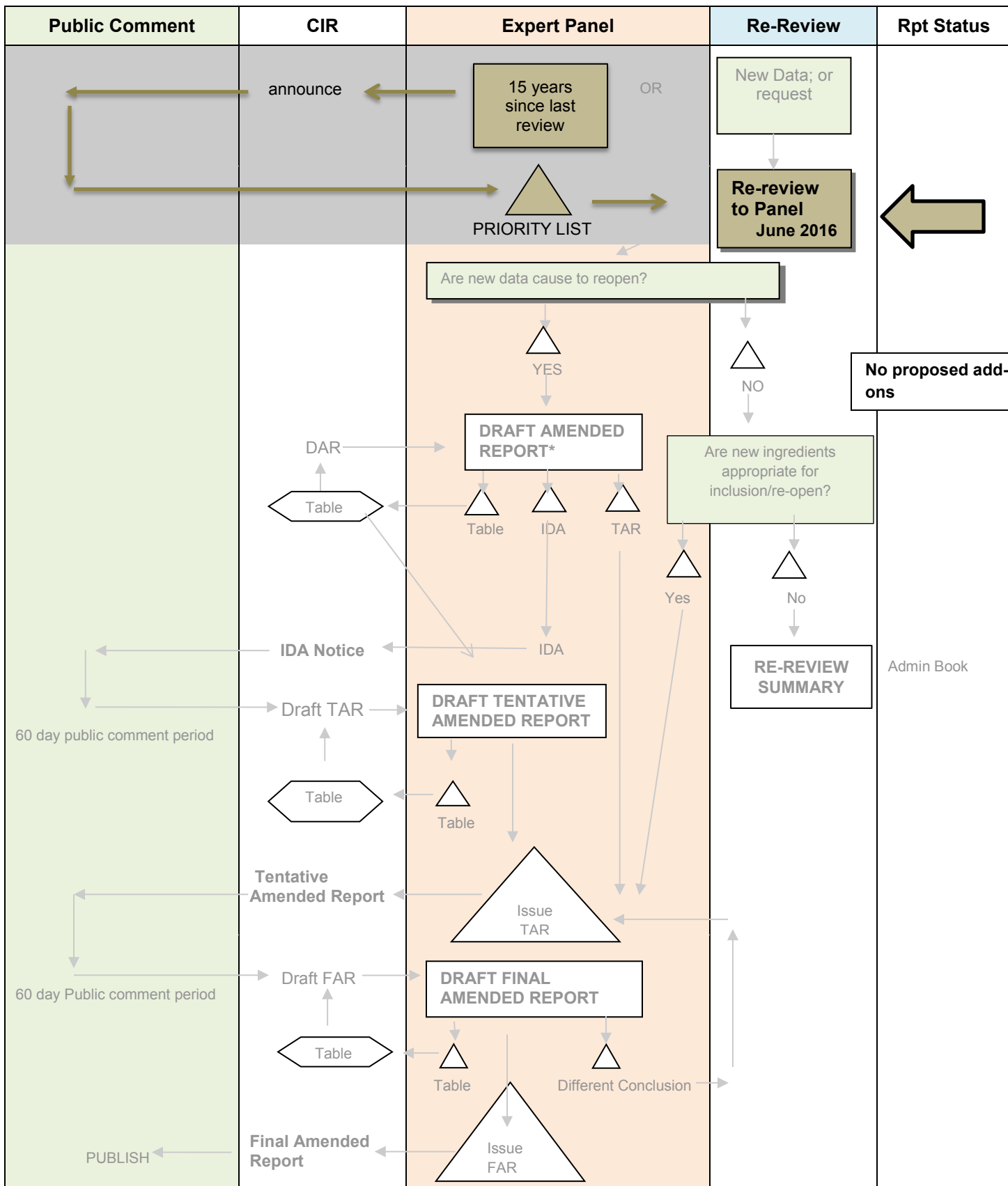
Minutes from the original discussions are provided for your review (*acidvi062016min*); the deliberations to evaluate safety during the original review were extensive. Also to facilitate your review, relevant information from the original review is included throughout the text of the report (indicated by italics), and the original Discussion is also included.

The Panel is now being asked to consider whether the new data present a reason to re-open the review. Or, do the data simply reaffirm the original conclusion, in which case the review would not be re-opened.

RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Acid Violet 43

MEETING June 2016



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

Acid Violet 43 - June 2016 - Monice Fiume

	Reported Use	Method of Mfg	Impurities	Dermal Penetration	ADME	Animal Tox - Acute, Dermal	Animal Tox - Acute, Oral	Animal Tox, Acute, Inhalation	Animal Tox - Rptd Dose, Dermal	Animal Tox, Rptd Dose, Oral	Animal Tox - Rptd Dose, Inhalation	Repro/Dev Toxicity	Genotoxicity	Carcinogenicity	Dermal Irr/Sens	Multicenter Studies	Ocular Irritation	Mucous Membrane Irr
new information - on Acid Violet 43	X		X	X	X					X		X	X		X	X	X	
new information - Ext. D&C Violet No. 2				X						X		X	X		X			
original report - Acid Violet 43	X	X																
original report - Ext. D&C Violet No. 2			X				X		X				X	X				

Acid Violet 43 RR

	CAS #	Use	PubMed	SciFinder	ChemID	NTIS	FDA	ECHA	IUCLID/ SIDS	WHO/ JEFCA	IARC	EU	SCCS	NICNAS	Web
Acid Violet 43	4430-18-6		67 hits/ 0 useful	98 hits		yes (1981 doc)	for Ext. Violet 2:	pre R		no	vol. 99	IV/90	yes	no	1 hit

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

ACID VIOLET 43 – MINUTES FROM ORIGINAL DELIBERATIONS

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 (Underarm) should be starred as an illegal use of Acid Violet 43.

Dr. Belsito 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.

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, and 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.

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

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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/Writer.

INTRODUCTION

In 2001, the Cosmetic Ingredient Review (CIR) Expert Panel (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: $\leq 18\%$ volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); $\leq 0.4\%$ water-insoluble matter; $\leq 0.2\%$ 1-hydroxy-9,10-anthracenedione; $\leq 0.2\%$ 1,4-dihydroxy-9,10-anthracenedione; $\leq 0.1\%$ *p*-toluidine; $\leq 0.2\%$ *p*-toluidine sulfonic acids, sodium salts; $\leq 1\%$ subsidiary colors; ≤ 20 ppm lead (as Pb); ≤ 3 ppm arsenic (as As); ≤ 1 ppm mercury as (Hg); and $\geq 80\%$ total color.¹ These are the specifications for Ext. D&C Violet No. 2, which is a certified colorant. 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. 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.

Because it has been 15 years since the safety assessment on Acid Violet 43 was published, the Panel is being asked to determine, based on data presented in this report, whether a re-review is warranted or the original conclusion can be reaffirmed. Much of the new information that is presented in this assessment was obtained from the European Commission (EC) Scientific Committee on Consumer Safety (SCCS) Opinion on Acid Violet 43.³

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 that are summarized refer to the certified dye. Additionally, the Discussion from the original report is included to indicate the rationale for the existing conclusion.

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 classed chemically as an anthraquinone color.² It conforms to the formula:

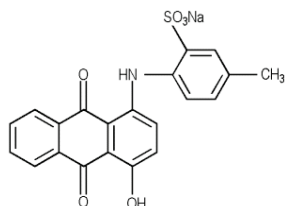


Figure 1. Acid Violet 43

Acid Violet 43 is the International Nomenclature of Cosmetic Ingredients (INCI) name for batches of this colorant that have not been certified.² To identify the certified colorant for labeling purposes in the United States (U.S.), the INCI name Ext. Violet 2 must be used. To identify the colorant allowed for use 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 431.4³ (Table 1). It does not absorb in the ultra-violet 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

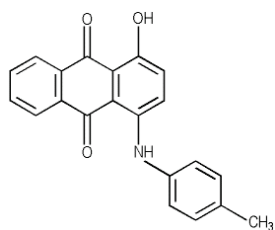


Figure 2. CI 60725²

Impurities/Composition

Acid Violet 43, as a non-certified color, tends to have more impurities than the certified color, Ext. D&C No. 2. 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; purity of Acid Violet 43 ranged 54.4-60.5%, and that of Ext. D&C Violet No. 2 ranged from 84-93.8% (Table 2).³

The specifications for Ext. D&C Violet No. 2, as described in the conclusion of the safety assessment published in 2001, have not changed. [21CFR74.2602a]

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this safety 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 reported to be used in oxidative and direct hair coloring products.⁴ 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.⁵ 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.⁶ 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.¹ 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 \leq 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 in order to be exempt from certain adulteration and color additive provisions of the U.S. Federal Food, Drug, and Cosmetic Act. In order to be exempt, the following caution statement must be displayed on all coal tar hair dye products:

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

Product labels shall also bear patch test instructions for determining whether the product causes skin irritation. 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.⁷ These authors concluded that, in its 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 performed on behalf of the European Society of Contact Dermatitis (ESCD) asserted that industry is focusing on predicting the risks from exposure to hair dyes by having millions of European consumers perform a self-test prior to each hair dyeing 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.⁸

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.³ The SCCS also stated that a sensitizing potential cannot be excluded. The SCCS further stated that Acid Violet 43 is also used as a colorant, but that use was not assessed in this Opinion. Sodium 4-[(9,10-dihydro-4-hydroxy-9,10-dioxo-1-anthryl)amino]toluene-3-sulphonate (CAS No, 4430-18-6; aka Acid Violet 43) is included in Annex III of the EC CosIng database (i.e., the List of Substances Which Cosmetic Products Must Not Contain Except Subject to the Restrictions Laid Down; III/291); according to regulation (EC) No 1190/2015, this substance is allowed as a hair dye substance in non-oxidative hair dye products at a maximum ready for use concentration of 0.5%.⁹

According to the EU Cosmetics Directive 88/667/EEC, regulation (EC) No 1223/2009, sodium 4-[(9,10-dihydro-4-hydroxy-9,10-dioxo-1-anthryl)amino]toluene-3-sulphonate (i.e., Acid Violet 43) is included in Annex IV of the EC CosIng database (i.e., List of Colorants Allowed in Cosmetics; IV/90), with the restriction that is not to be used in products applied to mucous membranes.¹⁰

TOXICOKINETICS STUDIES

Dermal Penetration

In Vitro

The percutaneous absorption of Acid Violet 43 (59.3% pure) was evaluated using human skin samples.³ The skin samples (thickness 380 ± 25 μ m) 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).³ 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 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 dose 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 “worst case scenario” 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 toxicokinetics study of Acid Violet 43 (59.8% pure) was performed as part of a bone marrow micronucleus test (described in the Genotoxicity section).³ In the toxicokinetics 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 bio-availability 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.*¹

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

Subchronic Toxicity Studies

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 dosed once daily by gavage for 13 wks with 0, 50, 200, or 800 mg/kg bw/day Acid Violet 43 (54.4% pure) in 5 ml/kg water.³ The dose rates 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 rates. Regurgitation occurred in all treatment groups. Colored urine, feces, hair, and extremities were observed at all dose rates 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 no-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).³ 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 dose rates 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 minutes, 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 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 dose rates 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.³ 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 dose rates 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.³ 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 incidental findings due to the lack of a dose-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.*¹

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 $\mu\text{g}/\text{plate}$ 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 $\mu\text{g}/\text{ml}$) with metabolic activation and in the 48-h treatment group at the highest concentration (272 $\mu\text{g}/\text{ml}$) 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.¹ 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 429.³ 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% hexylcinnamaldehyde in AOO) group were treated in the same manner. Lymphocyte proliferation was determined after a 2-day non-treatment period; no lymphoproliferation was observed at any test concentration. The highest stimulation index (SI) for the test article was 1.55 at the 15% test concentration. (An SI of ≥ 3 is regarded as positive.) No cutaneous reactions and no increases in ear thickness were 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 researcher commented that the highest concentrations of Acid Violet 43 tested (60.5% pure) 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.¹¹ 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 pre-treatment 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.³ 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 2007/2008.¹² 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 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.³

Absorption through the skin (A)	= 0.92 $\mu\text{g}/\text{cm}^2$
Skin Area surface (SAS)	= 580 cm^2
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 as a precursor in 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: $\leq 18\%$ volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); $\leq 0.4\%$ water-insoluble matter; $\leq 0.2\%$ 1-hydroxy-9,10-anthracenedione; $\leq 0.2\%$ 1,4-dihydroxy-9,10-anthracenedione; $\leq 0.1\%$ *p*-toluidine; $\leq 0.2\%$ *p*-toluidine sulfonic acids, sodium salts; $\leq 1\%$ subsidiary colors, ≤ 20 ppm lead (as Pb); ≤ 3 ppm arsenic (as As); ≤ 1 ppm mercury as (Hg); and $\geq 80\%$ 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 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 used 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 $\leq 0.1\%$ 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; the SCCS stated that Acid Violet 43 was not assessed in the opinion.

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 $0.11 \pm 0.06 \mu\text{g}$ active dye/ cm^2 (0.53 \pm 0.33% of the applied dose). In studies using porcine ear skin, the "worst case scenario" rates for skin absorption of Ext. D&C Violet No. 2 (95% pure) were estimated as 20.93 $\mu\text{g}/\text{cm}^2$ (approximately 1%) and 30.55 $\mu\text{g}/\text{cm}^2$ (approximately 0.6%) in two experiments.

In a toxicokinetics 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 NOEL 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 $\mu\text{g}/\text{plate}$ 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 $\mu\text{g}/\text{ml}$) 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 *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 researcher 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 (using 50% bioavailability) was calculated as 15,667.

DISCUSSION FROM ORIGINAL SAFETY ASSESSMENT¹

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: $\leq 18\%$ volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); $\leq 0.4\%$ water-insoluble matter; $\leq 0.2\%$ 1-hydroxy-9,10-anthracenedione; $\leq 0.2\%$ 1,4-dihydroxy-9,10-anthracenedione; $\leq 0.1\%$ p-toluidine; $\leq 0.2\%$ p-toluidine sulfonic acids, sodium salts; $\leq 1\%$ subsidiary colors; ≤ 20 ppm lead (as Pb); ≤ 3 ppm arsenic (as As); ≤ 1 ppm mercury (as Hg); and $\geq 80\%$ 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 irritation/sensitization reaction and allow them to avoid significant exposures.

TABLES**Table 1. Physical and chemical properties**

Property	Value	Reference
physical form	crystalline powder	3
Color	dark violet	3
molecular weight (g/mol)	431.4	3
water solubility (g/100 ml)	<1	3
other solubility (g/100 ml)	ethanol: <1 dimethyl sulfoxide: <1	3
log K _{ow}	3.1	3
UV Absorption (λ) (nm)	λ_{max} 253 nm, 570 nm	3

Table 2. Composition of Acid Violet 43 and Ext. D&C Violet No. 2³

	Units	Acid Violet 43			Ext. D&C Violet No. 2		
		Sample 1 [#]	Sample 2 [#]	Sample 3	Sample 1 [#]	Sample 2	Sample 3*
Titre^{##}	%	54.4	60.5	59.3	93.8	84	84
Impurities							
Acid Green 25		d	d	-	-	-	-
isomer of Acid Green 25		d	d	-	-	-	-
isomer of Acid Violet 43		d	d	-	-	-	-
1,4-dihydroxyanthraquinone	%	<0.25	<0.25	-	<0.2	-	-
1-hydroxy-9,10-anthracenedione	%	-	-	-	<0.2	-	-
p-toluidine	%	-	<0.1	-	<0.1	-	0.2
p-toluidine sulfonic acids, sodium salts	%	-	-	-	<0.2	-	-
Subsidiary colors	%	-	-	-	<1	-	-
Heavy metals (ICP-OES)							
Fe	mg/kg	-	270	-	-	-	-
Al	mg/kg	-	61	-	-	-	-
Sn	mg/kg	-	10	-	-	-	-
Cu	mg/kg	-	4	-	-	-	-
Mn	mg/kg	-	3	-	-	-	-
Ag, As, Ba, Bi, Cd, Co, Cr, Mo, Ni, Pb, Pd, Pt, Sb, Se, Ti, V, Zn	mg/kg	-	<1	-	As<3; Pb<20	-	-
Hg	mg/kg	-	<0.1	-	<1	-	-
Ash Content	g/100 g	25.2	25.9	-	-	-	-
Water content (K.F. method)	g/100 g	9.3	8.26	-	-	-	-
Starch content (expressed as glucose; HPIC)	g/100 g	-	26	-	-	-	-
Sulfate ions (expressed as Na ₂ SO ₄ ;HPIC)	g/100 g	0.43	6.5	-	-	-	*
Chloride ions (expressed as NaCl; potentiometry)	g/100 g	4.2	4.42	-	-	-	*
sum of volatile matter	%	-	-	-	<18	-	10
water-insoluble matter	%	-	-	-	<0.4	-	1
residual solvents (GC)	-	n.d.	n.d.	-	-	-	-
mixed oxides	%	-	-	-	-	-	1

[#]chemical characterization performed by nuclear magnetic resonance, infrared spectroscopy, and mass spectrometry

^{##} 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

Test Article	Purity	Concentration/Vehicle	Test System	Procedure	Results	Reference
IN VITRO						
Acid Violet 43	54.4%	170, 340, 680, 1360, and 2720 µg/plate; in water	<i>Salmonella typhimurium</i> TA1535, TA1537, TA98, and TA100 and in <i>Escherichia coli</i> WP2uvrA	Ames test, with and without metabolic activation; vehicle and valid positive controls were used	negative	3
Ext. D&C Violet No. 2	95%	31.35, 95, 316.35, 950, 2375, 4750 µg active dye/plate in DMSO	<i>S. typhimurium</i> TA1535, TA1537, TA98, and TA100 and in <i>E. coli</i> WP2uvrA	OECD 471; Ames test with and without metabolic activation; vehicle and valid positive controls were used	negative	3
Acid Violet 43	54.4%	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	human lymphocytes	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	24 h harvest – negative 48 h harvest - significant increase in aberrant cell frequency with the 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	3
Acid Violet 43	54.4%	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)	CHO cells	OECD 473; chromosomal aberration assay; 3- to 4-h exposure in the presence and absence 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	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	3
Ext. D&C Violet No. 2	95%	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	mouse lymphoma cell line L5178Y/TK+/-	OECD 476; mammalian cell gene mutation assay; 4 h treatment time; with and without metabolic activation; vehicle and valid positive controls were used 24 h treatment time, without metabolic activation	negative negative	3
IN VIVO						
Acid Violet 43	54.4%	0, 82 and 816 mg active dye/kg bw in water	3 male Wistar rats/group	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	negative	3
Acid Violet 43	54.4%	0 and 1088 mg active dye/kg bw in water	5 Swiss mice/sex/group	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	negative	3
Acid Violet 43	59.8%	0 and 2000 mg active dye/kg bw in purified water	5 Swiss mice/sex/group	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	negative	3

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

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Final Report on the Safety Assessment of Acid Violet 43¹

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

INTRODUCTION

Acid Violet 43 is an anthraquinone color that functions as a colorant in cosmetic formulations (Wenninger, Canterbury, 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

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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, Canterbury, and McEwen 2000).

Acid Violet 43 and Ext. D & C Violet No. 2 are also known as Cl 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, Canterbury, and McEwen 2000); Monosodium Salt of 1,5-Bis(*o*-Sulfo-*p*-Toluino) Anthraquinone (Hazleton Laboratories, Inc. 1968); Monosodium Salt of 1-Hydroxy-4-(Sulfo-*p*-Toluino)-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 (Hazleton 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 $>4.5 \times 10^3$ 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.

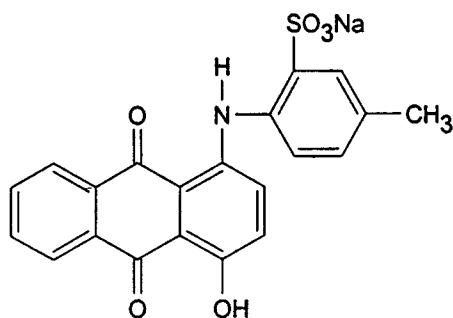


FIGURE 1
Acid Violet 43.

Impurities

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

USE

Cosmetic

Acid Violet 43 functions as a colorant in cosmetic formulations (Wenninger, Canterbury, and McEwen 2000). The product formulation data submitted to the FDA in 1998 stated that Acid Violet 43 was contained in a total of three cosmetic product formulations (FDA 1998) (Table 1).

Concentration of use values are no longer reported to the FDA by the cosmetic industry (FDA 1992). Historical product formulation and use data stated that Acid Violet 43 was used in 31 hair formulations at concentrations of $\leq 1\%$ (Table 2). No current concentration of use data are available.

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.

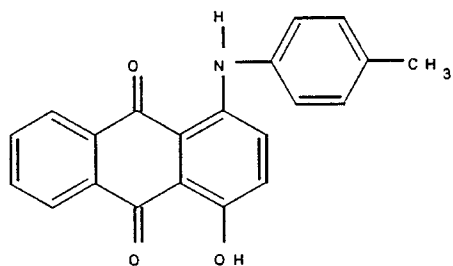


FIGURE 2
CI 60725.

TABLE 1
Product formulation and use data (FDA 1998)

Product category	Total no. of formulations in category	Total no. containing ingredient
Hair dyes and colors	1478	1
Hair rinses (coloring)	32	1
Deodorants (underarm)*	241	1
1998 total		3

*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.

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, Canterbury, 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 underarm deodorants is not; for that use, only Ext. D & C Violet No. 2 is permitted.

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.

TABLE 2
Historical product formulation and concentration of use data (FDA 1984)

Product category	0.1%–1%	0%–0.1%	Total
Hair dyes/colors (requiring caution statements)		25	25
Hair shampoos (coloring)	1	4	5
Hair bleaches		1	1
Total	1	30	31

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 violet 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 100 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 $\leq 1\%$.

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: $\leq 18\%$ volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); $\leq 0.4\%$ water-insoluble matter; $\leq 0.2\%$ 1-hydroxy-9,10-anthracenedione; $\leq 0.2\%$ 1,4-dihydroxy-9,10-anthracenedione; $\leq 0.1\%$ *p*-toluidine; $\leq 0.2\%$ *p*-toluidine sulfonic acids, sodium salts; $\leq 1\%$ subsidiary colors; ≤ 20 ppm lead (as Pb); ≤ 3 ppm arsenic (as As); ≤ 1 ppm mercury (as Hg); and $\geq 80\%$ 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: $\leq 18\%$ volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); $\leq 0.4\%$ water-insoluble matter; $\leq 0.2\%$ 1-hydroxy-9,10-anthracenedione; $\leq 0.2\%$ 1,4-dihydroxy-9,10-anthracenedione; $\leq 0.1\%$ *p*-toluidine; $\leq 0.2\%$ *p*-toluidine sulfonic acids, sodium salts; $\leq 1\%$ subsidiary colors; ≤ 20 ppm lead (as Pb); ≤ 3 ppm arsenic (as As); ≤ 1 ppm mercury (as Hg); and $\geq 80\%$ 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 irritation/sensitization reaction and allow them to avoid significant exposures.

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: $\leq 18\%$ volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts); $\leq 0.4\%$ water-insoluble

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

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²Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, N.W., suite 310, Washington, DC 20036, USA.

ACID VIOLET 43	06A - Hair Dyes and Colors (all types requiring caution statements and patch te	12
ACID VIOLET 43	06B - Hair Tints	1
ACID VIOLET 43	06C - Hair Rinses (coloring)	23
ACID VIOLET 43	06D - Hair Shampoos (coloring)	11
ACID VIOLET 43	06H - Other Hair Coloring Preparation	1

Concentration of Use by FDA Product Category – Acid Violet 43

Product Category	Maximum Concentration of Use
Hair dyes and colors	0.2-0.25%
Hair tints	0.32-0.35%
Hair rinses (coloring)	0.13%

Information collected 2015-2016

Table prepared February 16, 2016