Amended Safety Assessment of Basic Blue 99 as Used in Cosmetics

Status:	Draft Amended Report for Panel Review
Release Date:	February 10, 2023
Panel Meeting Date:	March 6-7, 2023

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina Burnett, MSES, Senior Scientific Analyst/Writer, CIR.

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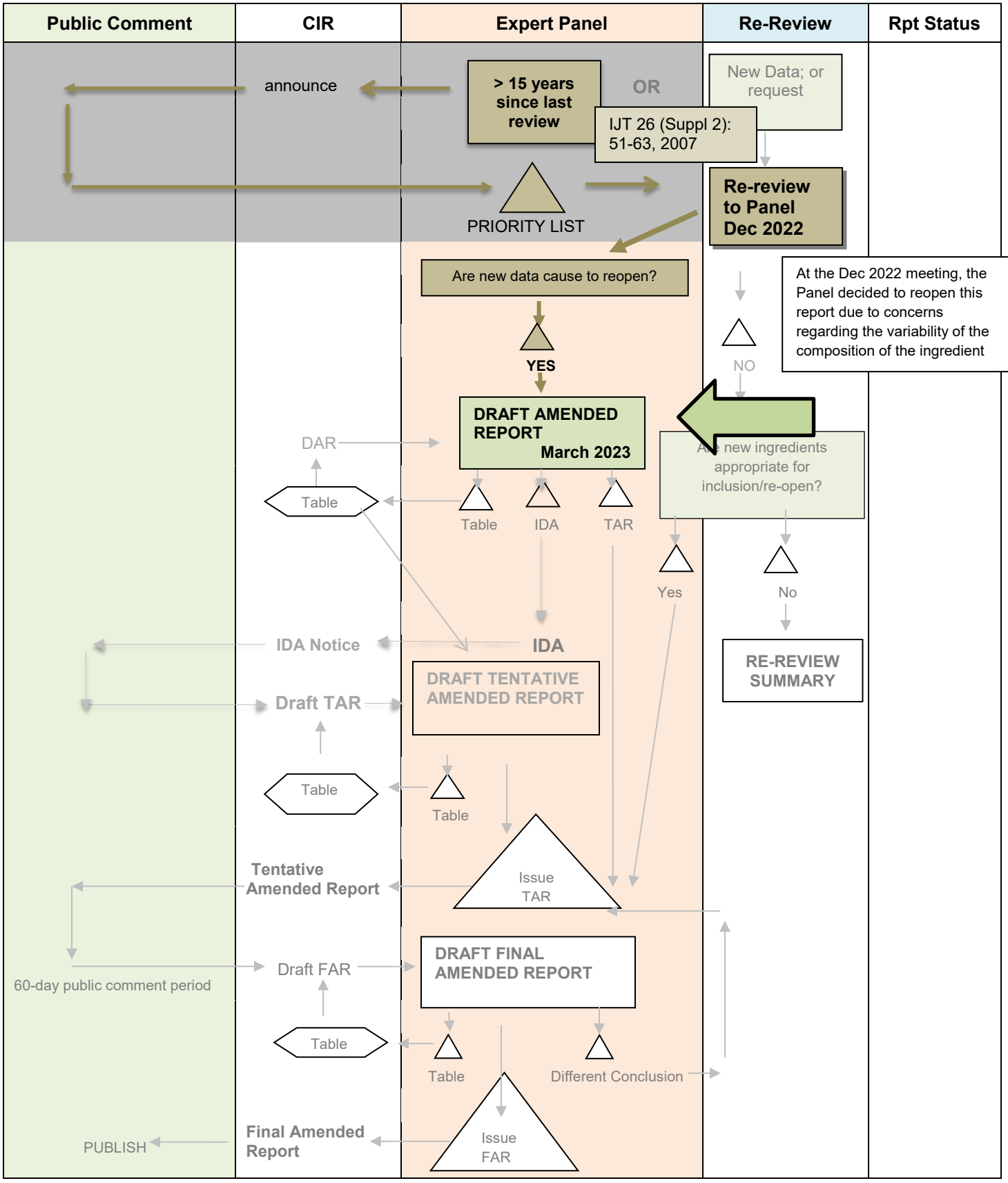
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Basic Blue 99

MEETING March 2023



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, MSES, Senior Scientific Analyst/Writer, CIR
Date: February 10, 2023
Subject: Amended Safety Assessment of Basic Blue 99 as Used in Cosmetics

The Panel previously reviewed the safety of Basic Blue 99 in an assessment that was published in 2007 (identified as *originalreport_BasicBlue99_032023* in the pdf document). In December 2022, the Panel determined that this safety assessment should be re-opened for re-evaluation due to concerns regarding the variability of the composition of the ingredient. Enclosed is the Draft Amended Report on the Safety Assessment of Basic Blue 99 as Used in Cosmetics (*report_BasicBlue99_032023*). In the original report, the Panel concluded that Basic Blue 99 is safe as a hair dye ingredient.

According to 2022 VCRP survey data, Basic Blue 99 has 38 reported uses; however, non-hair dye uses have been reported, including 1 use in nail polish and enamel and 6 uses in non-coloring hair products (*data_BasicBlue99_032023*). The results of the concentration of use survey provided by the Council in 2022 indicated this ingredient is used in hair dyes at a maximum concentration of 0.2%. When the original safety assessment was published in 2004, Basic Blue 99 was reported to have 51 uses in hair coloring products. In 2002, the maximum concentration of use for Basic Blue 99 in hair coloring products was reported to be 2%. As per the Panel's request at the December 2022 meeting, an updated use table format has been implemented. The frequency and concentration of use is presented both cumulatively by likely duration and exposure and individually by product category.

Since the December meeting, no new data have been submitted.

Additional supporting documents for this report package include a flow chart (*flow_BasicBlue99_032023*), report history (*history_BasicBlue99_032023*), a search strategy (*search_BasicBlue99_032023*), a data profile (*dataprofile_BasicBlue99_032023*), transcripts from the meeting at which the re-review was discussed (*transcripts_BasicBlue99_032023*), and the minutes from all the meetings at which Basic Blue 99 was discussed during the original review (*originalminutes_BasicBlue99_032023*).

If no further data are needed to reach a conclusion of safety, the Panel should formulate a Discussion and issue a Tentative Amended Report. However, if additional data are required, the Panel should be prepared to identify those needs and issue an Insufficient Data Announcement.

Basic Blue 99 History

2007– The CIR's Final Report on the Safety Assessment of Basic Blue 99 was published in the *International Journal of Toxicology*. The Panel had concluded that Basic Blue 99 is safe as a hair dye ingredient in the practices of use and concentration as described in this safety assessment.

December 2022 – Review of the available published literature since the original review was conducted in accordance to CIR Procedures regarding re-review of ingredients after ~15 years. The Panel re-opened the safety assessment for this ingredient due to concerns regarding the variability of the composition of Basic Blue 99.

Basic Blue 99 Data Profile* – March 2023 – Christina Burnett																														
				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Basic Blue 99	OX		OX	X	O	O		O			O			O	O	O			X	O			O	O			O		OX

* "X" indicates that new data were available in a category for the ingredient. "O" indicates data were reported in the original safety assessment.

Basic Blue 99

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Basic Blue 99	68123-13-7	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Search (from 2003 on)

PubMed

("Basic Blue 99") OR (68123-13-7[EC/RN Number]) OR (268-544-3 [EC/RN Number])-10 hits; 4 relevant

ECHA

Dossier for CAS # 68123-13-7 ("3-[(4-amino-6-bromo-5,8-dihydro-1-hydroxy-8-imino-5-oxo-2-naphtyl)amino]-N,N,N-trimethylanilinium chloride") was available. Safety test data in dossier either was from the original CIR report or it is described above. Additional chemical properties data is found in the table above.

Internet searches using trade names and other technical names. No relevant hits.

LINKS

Search Engines

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)

appropriate qualifiers are used as necessary
search results are reviewed to identify relevant documents

Pertinent Websites

- wINCI - http://webdictionary.personalcarecouncil.org
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

DECEMBER 2022 PANEL MEETING – RE-REVIEW

Belsito Team – December 5, 2022

DR. BELSITO: We first published a review of the safety of Basic Blue 99 in 2007 with the conclusion that this ingredient is safe as a hair dye ingredient in the present practices of use and concentration. Because it has been at least 15 years since it was published, the Panel should consider whether the safety assessment of Basic Blue 99 should be re-opened.

An exhaustive search of the world's literature was performed for studies dated 2003 forward. New case studies reporting allergic reactions to Basic Blue 99, the analysis of Basic Blue 99 by a new predictive dermal irritation assay, and additional chemical properties data were discovered. There are no restrictions from the EU; however, the EU SSCS determined it could not evaluate safety of Basic Blue 99 due to the variability of the ingredient's composition, which we can talk about.

We now have here current and historical reports of use data. Frequency of use has decreased slightly. However, there are non-hair dye uses that have been reported in 2022, including one in a nail polish and enamel and six in non --

DR. KLAASSEN: Can the microphone be turned on?

DR. BELSITO: Can you hear me now, Curt?

DR. KLAASSEN: Go ahead.

DR. BELSITO: Well, I'm just reiterating where we are with this Basic Blue 99. So, in 2022, it's being used at .2 percent, whereas before, it was reported as two percent. So, the question is whether to reopen it.

So, the old data, we had a negative 90-day oral, we had negative DART, mostly negative genotox, negative sensitization and irritation. No inhalation, but no aerosol use. And so, the question becomes how to deal with a nail and non-coloring hair products.

So, in terms of the non-coloring hair products, I just made a comment, is it possible that this was mistaken reporting? I mean, why would you put a dye in a non-coloring hair product?

MS. BURNETT: We've noticed in several of these hair dyes that we're looking at today, several of them have non hair dye use reported. It seems to be something with the VCRP data that we received this year. I don't know if a company is misreporting something or what's going on. We were hoping that FDA could clarify what's going on with that.

MS. KOWCZ: We'll have to look into it. I'm not sure exactly where it came from, but I'll have them check into it and get back to you.

MS. BURNETT: Thank you.

DR. BELSITO: So with these uses, I guess it's not approved as a colorant, so they're not under our purview to look at anyway. So, in terms of safety as used in a hair dye, the SCCS is saying they're not sure because the variability in composition, but I'm not seeing that. Where's the data that they looked at to come to that conclusion?

MS. BURNETT: They had several samples. So, they had three looks at this. And, like, the first time they had two samples and then they said that's -- impurity, I think, kind of has a wide range. And so, they asked for more data and then the next time they gave them like six samples. So, the composition data was all over the place. And then, like, the third was, like, nine sample, and there's still variability.

If you look at this, there is a wide purity range. And when the panel looked at this the first time, the panel noted that there is a wide variability, and it starts at like, 60 percent pure. The panel flagged it at that time. When you look at the minutes, someone in industry spoke up and said what the composition was, that there was, like, a lot of sugars and salts that form. And so, the composition is, I guess, hard to nail down because it can change.

I'm not sure, but it was noted when the panel first looked at it. And it was noted that it was sugars and salts.

DR. BELSITO: Yeah, that's what I have flagged. I said would these variations make a difference. Curious why the SCCS had issues. It's PDF Page 6. So this is Steiling (2002). I think that's how you pronounce his or her last name. Basic Blue 99, a mixture of about 70 percent Basic Blue, 20 percent sucrose and seven percent inorganic salts and four percent water. And the chromosphore component is predominantly about two-thirds, three isomers.

And in another description, it just says quaternary ammonium compounds that differ in the number of bromine atoms. So is that the variation that SCCS was seeing?

MS. BURNETT: I'm not entirely sure. I think their issue is that before they review tox data, they want a better idea of what it is so that when they receive tox data, they are certain that that tox data is on that ingredient and not some mixture of something. That's how I interpreted the last statement from them.

DR. RETTIE: I think in the European report, which I skimmed and can't bring up right now, they alluded to some 40 isomeric products, which is a lot more than the three that you mentioned. So, I wondered about that, whether that would be something that you would encounter if we went back at it and looked deeper. Because 40 is a lot more than three at the isomer level. I mean, it's basically variations on their chemical structure that we care about, not so much about salts and the rest of it.

DR. BELSITO: I did not look at the SCCS report, I just read the original. So in the SCCS report there were variations on the structure of what they found?

DR. SNYDER: No, they're isomers.

DR. BELSITO: The isomers?

DR. SNYDER: Reported, yeah.

DR. RETTIE: 40 of them.

DR. BELSITO: So I think we need to reopen this and look at their information? No?

DR. RETTIE: I thought so.

DR. BELSITO: I mean, from my standpoint, you know, I have nothing to say because it can sensitize all at once, but it doesn't.

MS. BURNETT: But what they have done is just all the analyses of HPLC data and like I said, it's all over the place. I can try to bring that in, but it's a lot of chromatographs and analyses charts.

DR. RETTIE: Well, if you take the SCCS report at face value -- and I think that's from 2017, so it's relatively new -- it's quite conceivable that there would be room for lots more isomeric products if, in fact, they did a decent job of the analysis, which I assume they did.

So for my money, just that on its own was enough for me to think we need to reopen it and take a closer look. I wish I could pull up the SCCS report right now just to be sure of what I'm seeing.

MS. BURNETT: I can see if I can.

DR. RETTIE: You know, that 40 isomeric product is in the summary, so that should be easy to find.

DR. BELSITO: I mean, this is your bailiwick, Allan. You know, if you think it needs to be opened, then let's reopen it and take a look.

DR. RETTIE: Well, you've got a structure here with an aromatic amine on it and there's 40 things that look like that. I mean, we should do due diligence and reopen it.

DR. BELSITO: Okay. So at this point in our discussion, we clearly point out that non-hair uses are not within our purview. And that it has not been approved as a colorant, so these nail enamels and non-coloring hair products are misbranded. And given the apparent variations reported by the SCCS in composition, we want to reopen to look at composition and then other data may be needed, depending upon what we see. I don't know what other data we can specify without looking at that report.

MS. BURNETT: Right there. Sorry, we just pulled up the report.

DR. RETTIE: So, there's a raft of isomeric products that they saw across the six batches that they analyzed. Yeah, isomeric, they're all analogues or part hydroxy analogues? It looks like a more complex mixture than I was given the impression that was done before.

DR. BELSITO: Okay. So I have discussion, non-hair dye use not our purview and product's misbranded as not an FDA-approved coloring. And reopen to assess the composition variability that's been noted by the SCCS. And additional data may be needed depending upon that variability.

DR. RETTIE: All good. Sounds about right. I mean, I've also got a note about new irritation reports as well. I'm not sure how extreme that was or if that'll all get rolled in.

DR. BELSITO: And then I just had a question here on PDF Page 3, Christina. The predictive dermal irritation, there's not a concentration given. Basic Blue has more pronounced necrotic death than 2-dimensional cultures while apoptosis was observed in 3-dimensional. It doesn't give the concentration.

MS. BURNETT: I'm not sure there was one. I'll check when we present the reopened document.

DR. BELSITO: Okay. And then FDA is going to look into possible misrepresentation of the non-hair coloring dyes. Okay.

DR. RETTIE: Now, I get the impression that impurities are high, at least at the level of the organic content. When they report 60 percent impurities, they're adding in all the inorganic components as well. So maybe less variability. Oh well, maybe not. We have to see it. I think we have to see it.

Cohen Team – December 5, 2022

DR. COHEN: So, we'll start with Basic Blue 99. In 2007, the panel reviewed this ingredient with a conclusion that it was safe as a hair dye ingredient in present practices and use. It's been 15 years and the question is should we reopen Basic Blue 99.

An exhaustive search was done and there was some cases of dermatitis and there was a new study predicting dermal irritation. The max use is overall less in 2002, and there was a note of one use in nail polish which is not an authorized use. So, for the panel, any comments about a desire to reopen this? Susan?

DR. TILTON: So, I had noted just due to the decreased frequency of use and concentration of use, and that the outcomes from the included new data were really not different than previously reported, that there would be -- that a recommendation for no rereview.

DR. COHEN: Tom?

DR. SLAGA: Yes. The only comment that I didn't quite understand in the reading, related to this rereview, is that the European commission scientist stated that they couldn't review it because of the variability of ingredients. I didn't quite -- couldn't find anything to support that and I'm just checking with the chemist. Other than that, it was safe before and I would say do not reopen it. Same conclusion.

DR. COHEN: David?

DR. ROSS: Yeah, Tom, I think it was variability in the composition of the batches, I think, that they commented on. And yeah, I agree with Susan. I mean, the uses went, I think, from 51 to 38, the concentrations went down tenfold from two percent. So, my conclusion was no, don't reopen it.

DR. COHEN: Okay, so, we are unanimous on not reopening.

MS. BURNETT: Just for the summary. You'd like me to point out that the non-hair dye uses are not --

DR. COHEN: Not authorized.

MS. BURNETT: Not authorized.

DR. COHEN: Not authorized. What's the right term?

DR. HELDRETH: It's an unlawful use.

DR. COHEN: It's an unlawful use?

DR. HELDRETH: Yeah. Colorant cosmetics ingredients need to be approved by the FDA before they go on the market. If there's a hair dye exemption, a coal tar hair dye exception, that puts the purview of assessing these oxidative hair dyes in this panels court. But as soon as you step away from hair dye use, that exemption falls and manufactures must get approval from FDA for a color such as this before it can be used.

DR. COHEN: Okay. I'm just making some notes for tomorrow. All right, any other comments on Basic Blue?

DR. BERGFELD: Are you suggesting that we put in the European decision and why into the review summary?

DR. COHEN: I wasn't, but anyone --

DR. ROSS: Does it make a difference if we do?

DR. BERGFELD: No.

DR. COHEN: It seemed vague.

DR. BERGFELD: I think if you put it in your document, it's a major entry on what the Europeans are doing.

MS. BURNETT: I would note when the panel reviewed this originally, there was a question on the compositions back then because the purity levels are pretty low in this. That according to the minutes, someone from industry came and said that there's a variability in this and that the variability is because there are sugars and salts produced in the dye.

So, the panel was aware, during the first review, that the composition was kind of inconsistent. And I think that's what the European union was also saying, the inconsistency on the composition. And until they get -- you know, they want like a concrete composition. I'm not sure they're ever going get it just based on the chemistry of it.

DR. BERGFELD: Could that be repeated in a sentence? I think that would be appropriate. It'll be always a question why did they not and why are we, yes, leaving it as it is.

DR. ROSS: It's okay with me. I think it's a good idea.

DR. COHEN: The other report says 60 percent has dye content and the rest is sugar and salt.

DR. HELDRETH: All right, we'll draft a sentence to put in the rereview summary and then you all will get an opportunity to review that and determine if it fits.

DR. COHEN: Okay. We're going to move onto HC Yellow Number 5.

Full Panel – December 6, 2022

DR. COHEN: The Expert Panel first published a review of safety of Basic Blue 99 in 2007 with conclusion that it's safe as a hair dye ingredient. It's been at least 15 years since that report. There were some new case studies that came to our attention. No restrictions in Europe. The max use is overall less than in 2002. There's one notation of its use in nail polish, which we would comment as an adulterant and unlawfully used. Our motion is do not reopen.

DR. BERGFELD: Is there a second, or a comment?

DR. BELSITO: We actually felt that we needed to reopen to assess the composition variability that was noted by the SCCS. In addition, additional data might be needed after we look at that variability.

DR. COHEN: We've seen, in some of the other reports, broad purity statements. And, we weren't sure it was really going to change the report. But, okay, if you want more on that.

DR. BERGFELD: You want to rescind your motion?

DR. COHEN: So, we don't need to put a data need; we're just going to reopen it, is what you guys are suggesting, we reopen?

DR. BELSITO: Yes. Reopen to look at the SCCS report and the variability. Go ahead, Allan, I'm sorry.

DR. RETTIE: Well, at least I was struck by the comment that the variability from that report reflected possibility that you had a mixture of 40 chemical analogues. I mean, that just got my attention, as well as a bunch of isomers in there, which we ought to get at. But 40 chemical analogues seemed like a lot.

DR. COHEN: So, we'll reopen -- because the whole report will be reopen, it's not just going to be for one thing. So, I will amend my motion to reopen.

DR. BELSITO: Seconded.

DR. BERGFELD: Seconded. Any further discussion regarding reopening this ingredient? Seeing none, I'll assume it's unanimous.

BASIC BLUE 99

Full Panel – September 10-11, 2002

An SLR on Basic Blue 99 was announced on May 17, 2002. Unpublished data and comments were received during the 90-day comment period.

Dr. Belsito stated that Basic Blue 99 is a coal-tar containing hair color, and, as such, carries a warning label relating to sensitization potential and the need for patch testing. He noted that his Team had expressed concern over data in the report indicating that Basic Blue 99 is 63% pure and that the remaining 37% of the composition is not listed. However, at yesterday's Team meeting, it was pointed out that the remaining 37% consists of sugars that are present to help disperse the compound.

Dr. Belsito said that after receiving the information on composition with the assurance that it would be incorporated and properly cited in the report text, his Team concluded that Basic Blue 99 is safe as used in cosmetic products.

The Panel voted unanimously in favor of issuing a Tentative Report with a safe as used conclusion on Basic Blue 99.

Full Panel – February 6-7, 2003

Dr. Marks recommended that the Final Report on Basic Blue 99 be tabled until the updated epidemiology data on hair dyes (promised by Dr. McEwen) is made available later this year. However, he noted that his Team is of the opinion that a safe as used conclusion on Basic Blue 99 will be reached.

Dr. Belsito said that his Team determined that Basic Blue 99 is safe as used in cosmetic products, based on current data, and that a Final Report should be issued at this meeting. He noted that rather than delaying issuance of the Final Report at today's meeting, the safety of this ingredient in cosmetics could be reassessed after the epidemiology study has been made available and, if necessary, the report could then be reopened.

Dr. McEwen said that it would be appropriate to hold the document until the epidemiology data on hair dyes are available. He noted that at least ten years have passed since the last update. Dr. McEwen said that it is expected that the epidemiology report will be made available prior to the September 8-9 Panel meeting this year.

Dr. Bergfeld recommended that the Panel's conclusion should state that Basic Blue 99 is safe as used in hair dyes. She said that since it is the Panel's intention to conclude that this ingredient is safe as used, the wording of the conclusion should be agreed upon at the September Panel meeting.

Dr. Marks said that the wording of the existing tentative conclusion on Basic Blue 99 prompted his Team to question the language that is used in CIR report conclusions. The conclusion is stated as follows: On the basis of the animal and clinical data included in this report, the CIR Expert Panel concludes Basic Blue 99 is safe as used in cosmetic formulations. Dr. Marks noted that concentration is not mentioned, and that the Panel's expert judgement is not taken into consideration.

Dr. Belsito said that his Team suggested that the conclusion should begin with the phrase on the basis of the data reviewed in the safety assessment.

Dr. Marks stated that the use of this phraseology may be interpreted as a tendency to overlook the experience and assessment of experts on the Panel. He noted the possibility of another panel of experts arriving at a different conclusion after reviewing the same data set.

Dr. McEwen also disagreed with this phraseology. He said that the Panel's conclusions on the safety of ingredients in cosmetics is not based only on the data reviewed, but, also, on the expertise and professional judgement of the Expert Panel.

Dr. Belsito agreed that a CIR report conclusion is based on the available data as well as the Panel's expertise.

Dr. Snyder said that the report conclusion should be very succinct and not capture the Panel's collaboration on points, which, more appropriately, should be included in the report discussion.

Dr. Marks said that an appropriate boilerplate conclusion needs to be developed, considering that the wording of CIR report conclusions varies greatly. He then read the following two proposals for boilerplate conclusions (provided by Drs. McEwen and Andersen):

1. The CIR Expert Panel concluded that ingredient name is safe as a cosmetic ingredient in the present practice of use and concentration as reflected in this report.
2. The CIR Expert Panel concluded that ingredient name is safe as a cosmetic ingredient (specific type stated, e.g. hair dye) in the present practices and concentration of use as described in this safety assessment.

Dr. Andersen said that the key feature of both wordings is that the concept of present practice of use and concentration is captured and that the entire report or safety assessment is identified as the basis for the conclusion. Additionally, the latter conclusion allows the specific type of ingredient to be flagged, if appropriate.

Dr. Bergfeld said that the two proposed conclusions will be considered at the September 8-9, 2003 Panel meeting. She added that it would be helpful if Dr. Andersen would provide the Panel with the variety of different conclusions that the Panel has arrived at in the past for consideration at this meeting.

Dr. Belsito wanted to know whether a change in the conclusion to indicate that Basic Blue 99 is safe as used in hair dyes (i.e., a more restrictive conclusive) would be considered substantive, and, with this in mind, whether a public comment period on this revised conclusion would be necessary.

Dr. McEwen noted that, according to the CIR report, Basic Blue 99 is only used in hair dyes, and that the qualification safe as used in hair dyes would not require another public comment period.

The Panel voted unanimously in favor of tabling the Expert Panel's report on Basic Blue 99 until the September 8-9, 2003 Panel meeting, pending the current hair dye epidemiology report that will be provided by industry. The Panel also unanimously confirmed its earlier conclusion that this ingredient is safe, though the exact wording of the conclusion has not been determined.

Dr. Andersen said that in addition to stating that the report on Basic Blue 99 was tabled at this meeting, the following should also be announced: (1) the Panel's general view that Basic Blue 99 is safe as a hair dye ingredient and (2) explanation of the remainder of the Panel's thinking in terms of a revised wording of the conclusion.

Dr. Andersen outlined the Panel's future action on Basic Blue 99 as follows: (1) At the September 8-9, 2003 Panel meeting, the Panel will be provided with a hair dye epidemiology summary and a CIR version of a new boilerplate conclusion (i.e., uniform language that will be used to describe the Panel's views of the epidemiology data to date). (2) This language, along with supporting documents, will be considered for formal approval by the Panel. (3) The public may want to comment on this new information (i.e., the Panel's view of the current state of epidemiology). At the end of the comment period, the Panel would arrive at a final decision based on comments that have been received. It would then be appropriate to insert the new information into the CIR report and consider it an editorial change.

Dr. Andersen said that under the preceding proposed plan, it would not be necessary to issue another Tentative Report (with 90-day public comment period) on Basic Blue 99. However, the public will have an opportunity to comment on the new epidemiology section of the report, and, at the end of the comment period, this information will be incorporated into the report.

Dr. Marks proposed the following editorial change for the Tentative Report on Basic Blue 99: (1) deletion of the following paragraph from the report discussion: Based upon the UV spectrum of Basic Blue 99 the Panel concluded that additional phototoxicity data were needed.

Full Panel – March 15-16, 2004

Dr. Belsito stated that this ingredient is one of the hair dyes that was placed on hold pending approval of the hair dye epidemiology boilerplate.

The Panel voted unanimously in favor of issuing a Final Report with the following conclusion: Basic Blue 99 is safe as a hair dye ingredient in the practices of use and concentration as described in this safety assessment.

Dr. Belsito said that it should be stated in the report introduction that Basic Blue 99 is a direct, non-oxidative hair colorant and that the first paragraph of the report summary should begin with this statement. He also recommended removal of the last paragraph of the report discussion to the report summary (after 1st paragraph).

Amended Safety Assessment of Basic Blue 99 as Used in Cosmetics

Status: Draft Amended Report for Panel Review
Release Date: February 10, 2023
Panel Meeting Date: March 6-7, 2023

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D. This safety assessment was prepared by Christina Burnett, MSES, Senior Scientific Analyst/Writer, CIR.

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ABBREVIATIONS

3D	three-dimensional
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
Dictionary; wINCI	web-based *International Cosmetic Ingredient Dictionary and Handbook*
DMSO	dimethyl sulfoxide
EHE	Equivalent Human Epidermis
FDA	Food and Drug Administration
LD_{50}	median lethal dose
LLNA	local lymph node assay
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide
NOEL	no-observable-effect-level
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
SCCS	Scientific Committee on Consumer Safety
TUNEL	terminal deoxynucleotidyl transferase nick-end labeling
TG	test guideline
US	United States
VCRP	Voluntary Cosmetic Registration Program

INTRODUCTION

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), Basic Blue 99 is reported to function in cosmetics as a hair colorant and a hair conditioning agent.[1] Basic Blue 99 was previously reviewed by the Expert Panel for Cosmetic Ingredient Safety (Panel) in a safety assessment that was published in 2007.[2] At that time, the Panel concluded that Basic Blue 99 "is safe as a hair dye ingredient in the present practices of use and concentration." In accordance with its Procedures, the Panel evaluates the conclusions of previously-issued reports approximately every 15 years, and it has been at least 15 years since this assessment has been issued. In December 2022, the Panel determined that this safety assessment should be re-opened for re-evaluation due to concerns regarding the variability of the composition of the ingredient.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an exhaustive search of the world's literature. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties. Excerpts from the summaries of the previous report on Basic Blue 99 are disseminated throughout the text of this re-review document, as appropriate, and are identified by *italicized text.*

CHEMISTRY

Definition and Structure

According to the *Dictionary*, Basic Blue 99 (CAS No. 68123-13-7) is the naphthoquinoneimine color that conforms to formula in Figure 1.[1]

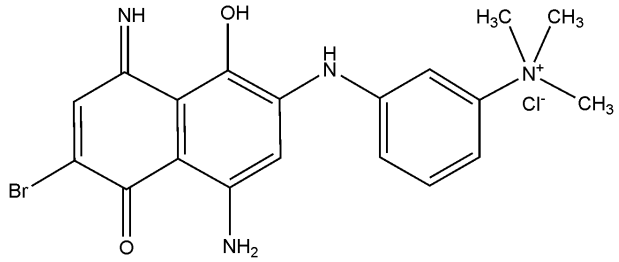

Figure 1. Basic Blue 99

This ingredient is a non-oxidative, temporary to semi-permanent, direct-dye hair colorant. Thus, unlike oxidative hair dyes, no oxidation or coupling is needed before use.

Chemical Properties

Chemical properties for Basic Blue 99 are summarized in Table 1. Basic Blue 99 has a formula weight of 451.8 g/mol and is soluble in water at 25 ºC and 60 ºC, while slightly soluble in isopropyl alcohol at 60 ºC and insoluble therein at 25 ºC.[2] Basic Blue 99 is also reported to be soluble in ethanol.

Method of Manufacture

Method of manufacturing data were not found in the published literature, and unpublished data were not submitted.

Composition and Impurities

According to a supplier, Basic Blue 99 must be at least 63% pure Basic Blue 99 in the color mixture and have no more than 100 ppm of iron.[2] Another supplier of Basic Blue 99 has a specification of 60.7% dye content, 25.7% sugar content, 11.8% inorganic salts, and 1.8 % volatile matter/water crystallization. The majority of the additional contents of hair dye are understood as being part of the color and not undesirable chemical impurities.

The Scientific Committee on Consumer Safety (SCCS) reported that the purity of Basic Blue 99 (as cation) was 58.0-70.0% between batches.[3] Several isomeric forms and a mixture of up to 40 chemical analogues, including subsidiary colors, may comprise a single batch of Basic Blue 99. Aside from the isomers and the chemical analogues, inorganic impurities have been quantified as the following: lead (< 20 ppm), antimony and nickel (< 10 ppm), arsenic and cadmium (< 5 ppm), and mercury (< 1 ppm).

USE

Cosmetic

The safety of the cosmetic ingredient addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics, and does not cover their use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2022 VCRP survey data, Basic Blue 99 has 38 reported uses; non-hair dye uses have been reported, including 1 use in nail polish and enamel and 6 uses in non-coloring hair products (Table 2).[4] The results of the concentration of use survey provided by the Council in 2022 indicated this ingredient is used in hair dyes and colors at 0.2%; concentrations of use were not reported for non-hair dye formulations.[5] When the original safety assessment was published in 2004, Basic Blue 99 was reported to have 51 uses in hair coloring products.[2] In 2002, the maximum concentration of use for Basic Blue 99 in hair coloring products was reported to be 2% in hair tints.

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

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

Product labels shall also bear patch test instructions for determining whether the product causes skin irritation. However, whether or not patch testing prior to use is appropriate is not universally agreed upon. The Panel recommends that an open patch test be applied and evaluated by the beautician and/or consumer for sensitization 48 h after application of the test material and prior to the use of a hair dye formulation. Conversely, a report in Europe suggests that self-testing has severe limitations, and may even cause morbidity in consumers.[6,7] Hair dye products marketed and sold in the US, though, must follow the labeling requirements established by the Food, Drug, and Cosmetic Act.

In the European Union, the most recent opinion by the SCCS stated the committee could not evaluate the safety of Basic Blue 99 due to the large variability of the ingredient's composition between different batches.[3] Toxicological data previously submitted to the SCCS "do not relate to the material specifications provided for the current assessment" and "the safety assessment of Basic Blue 99 will require a clear well-defined set of specifications for the composition of the material intended to be used in cosmetic products as well as supporting toxicological data relating to a representative composition." Under European regulations for cosmetic ingredients, however, there are no restrictions for use of Basic Blue 99.[8]

TOXICOKINETIC STUDIES

Skin Penetration/Dermal Absorption

In rat studies of aqueous solutions of [^{14}C] labeled Basic Blue 99 (up to 1%) to assess skin penetration potential, most of the test material was recovered in rinse water, patches, or in the treated area of the skin.[2] Levels of this radiolabel were low in the urine and feces. It was determined there was very low percutaneous absorption of Basic Blue 99 in rats. In human volunteers treated topically (to hair) with a hair setting solution containing [^{14}C] labeled Basic Blue 99 (40% aqueous solution containing 0.1% of other dyes), levels of this radiolabel in the urine were less than 0.1% of the applied dose.

Absorption, Distribution, Metabolism, and Excretion

Aqueous solutions of [^{14}C] labeled Basic Blue 99 was poorly absorbed when administered orally and intraperitoneally in rats.[2] Most of the radiolabel was recovered in the feces within the first 24 h. The radiolabel was also recovered at lower quantities in the urine, and was barely detectable in expired air, blood, and carcass, when measured.

Additional toxicokinetic studies were not found in the updated literature search, and unpublished data were not submitted.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

In acute oral toxicity studies, the median lethal dose (LD_{50}) values of Basic Blue 99 were 2700 mg/kg bw in mice and between 1000 mg/kg bw and greater than 2000 mg/kg bw in rats.[2]

Additional acute toxicity studies were not found in the updated literature search, and unpublished data were not submitted.

Subchronic Toxicity Studies

In a subchronic study, mice (10 per dose group) that received Basic Blue 99 at up to 500 mg/kg bw/d in the diet for 90 d did not give any indications of cumulative toxicity.[2] *No deaths occurred. Discoloration of organs involved in the elimination of Basic Blue 99 from the animals was noted. A 90-d oral toxicity study of rats (10 animals per sex per dose group) that received up to 360 mg/kg bw Basic Blue 99 via gavage also found no indications of cumulative toxicity. The no-observable-effect-level (NOEL) was between 180 to 360 mg/kg bw/d (range due to an increase in the dose after 8 wk).*

Additional repeated-dose toxicity studies were not found in the updated literature search, and unpublished data were not submitted.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Basic Blue 99 (50 mg/kg/d) administered by gavage did not cause embryotoxic or teratogenic effects in 29 rats.[2] *No mortalities were observed in the dams that had been treated on days 6 through 15 of gestation and no cumulative toxicity was observed.*

Additional developmental and reproductive toxicity studies were not found in the updated literature search, and unpublished data were not submitted.

GENOTOXICITY STUDIES

Basic Blue 99 (tested at up to 2500 µg/plate) was mutagenic with and without metabolic activation in the Ames test using Salmonella typhimurium, producing both reverse and frameshift mutations.[2] *However, Basic Blue 99 did not induce mutations using Escherichia coli at up to 100 µg/ml. Basic Blue 99 was not genotoxic in one chromosome aberration test in Chinese hamster V79 cells when tested in dimethyl sulfoxide (DMSO) at up to 10 µg/ml without metabolic activation and at up to 250 µg/ml with metabolic activation; however, this ingredient was considered to be clastogenic with or without metabolic activation in another chromosome aberration test in the same cell system using a deionized water vehicle and at doses up to 1.5 µg/ml without metabolic activation and up to 45.0 µg/ml with metabolic activation. Basic Blue 99 was not genotoxic in unscheduled DNA synthesis assays using rat hepatocytes (at up to 1000 mg/kg bw) or in micronucleus assays using mouse bone marrow cells (at up to 1500 mg/kg bw).*

Additional genotoxicity studies were not found in the updated literature search, and unpublished data were not submitted.

CARCINOGENICITY STUDIES

Carcinogenicity studies were not were not included in the original report and found in the updated literature search, and unpublished data were not submitted.

DERMAL IRRITATION AND SENSITIZATION

Irritation

Undiluted Basic Blue 99 (0.5 g/in^2) produced no observable reactions in one 24-h dermal irritation study with 6 rabbits, but was considered mildly irritating in another study with 6 rabbits.[2] *The primary irritation index in the second study was calculated to be 0.2.*

In Vitro

In an evaluation of an Equivalent Human Epidermis (EHE) three-dimensional (3D) model that utilized immortalized keratinocytes (HaCaT cells), the potential toxic effects induced by Basic Blue 99 (> 63% pure) were studied and compared to those observed in monolayer cultured cells.[9] Comet assay results indicated Basic Blue 99 (up to 35 µg/ml) did not induce DNA damage in the monolayer cell culture nor in the 3D cell culture. To measure cell viability measurement, the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay (Basic Blue 99 tested at up to 50 µg/ml) and the terminal deoxynucleotidyl transferase nick-end labeling (TUNEL) assay (Basic Blue 99 tested at up to 500 µg/ml) were performed in monolayer culture and in the EHE model, respectively. Basic Blue 99 had more pronounced necrotic death in monolayer cultures, while apoptosis was observed in a 3D environment. Based on the data generated from the EHE 3D model, the authors stated a decrease in the keratinocytes viability was expected after human dermal exposure to Basic Blue 99, which could result in dermal irritation. However, it should be noted this study was published before the Organisation for

Economic Co-operation and Development (OECD) test guideline (TG) 439 on reconstructed human epidermis 3D models was finalized. The study does not meet the OECD TG requirements as the viability measurement was performed within the incorrect timeframe with inadequate cell incubation time and the wrong assay was used to measure cell viability.

Sensitization

No delayed contact hypersensitivity was observed in a guinea pig maximization test (n = 10) of aqueous Basic Blue 99 induced at 75 % and challenged at 25%.[2] In a local lymph node assay (LLNA) using groups of 4 mice, Basic Blue 99 was not considered sensitizing when tested at up to 25% in twice-distilled water; however, in another study using groups of 5 mice, Basic Blue 99 may induce sensitization when tested at up to 2.0% in DMSO. The results of the latter assay had responses statistically significantly greater than the vehicle control, but no test/control ratios were greater than 3. A positive response was also observed in the 0.25% group, but the difference was not statistically significant when compared to the vehicle control. No irritation was observed in the mice treated with the test material in these LLNAs. Basic Blue 99 (concentration not reported) was not a sensitizer in a modified repeated-insult patch test in 54 volunteers.

Additional sensitization studies were not found in the updated literature search, and unpublished data were not submitted.

OCULAR IRRITATION STUDIES

Basic Blue 99 (0.5%) did not cause ocular irritation in 3 rabbits.[2] Discoloration was noted.

Additional ocular irritation studies were not found in the updated literature search, and unpublished data were not submitted.

CLINICAL STUDIES

Case Reports

Case reports have documented positive patch test results to 1% Basic Blue 99 in 3 patients.[2] One of the patients presented with an immediate reaction, while the other two were delayed.

A 57-yr-old woman with an employment history as a hairdresser presented with eczema on the hands and feet.[10] Previous patch testing was positive for *p*-phenylenediamine, nickel, chromium, cobalt, and colophonium. The patient also reported a history of severe itching on the hands and in the ears, accompanied by a "bad taste" in the mouth following use of a name-brand hair-coloring formulation. The patient underwent additional patch testing, which was positive for *p*-toluenediamine, methyldibromo glutaronitrile, and several extracts of "hypoallergenic leather." Prick testing with the hair-coloring formulation resulted in a strong positive reaction within 15 min of the test. Additional prick tests with the ingredients of the hair-coloring formulation resulted in strong reactions to 1% Basic Blue 99 aq. (+++, > histamine) and 1% Basic Brown 17 aq. (++, = histamine). A repeated prick test several months later resulted in the same results, but a patch test with the ingredients of the hair-coloring formulation was negative. A prick test with components identified by thin-layer chromatography from the formulation indicated that the patient may be sensitized to some impurities present.

In another case report, a 56-yr-old woman with a history of asthma experienced anaphylaxis following use of a semi-permanent, non-oxidative hair dye product.[11] A prick test with the hair dye product, which contained Basic Blue 99, was positive. Another prick test was performed with 0.1% Basic Blue 99 aq., which yielded a positive result with pseudopod development. Prick tests with Basic Blue 99 at 0.01% aq. and 0.001% aq. resulted in a slight reaction and a negative result, respectively.

A case study review of contact urticaria cases related to cosmetic and industrial dyes included a discussion regarding Basic Blue 99.[12] A case study summarized in the original CIR safety assessment of Basic Blue 99 was analyzed to further categorize the adverse reaction observed as a potential immunologic contact urticaria instead of a general contact urticaria. The authors noted that dyes like Basic Blue 99 may have multiple impurities, whose immunogenicity has not been studied.

HAIR DYE EPIDEMIOLOGY

Hair dyes may be broadly grouped into oxidative (permanent) and direct (temporary or semi-permanent) dyes. The oxidative dyes consist of precursors mixed with developers to produce color, while direct hair dyes consist of preformed colors. Basic Blue 99 is reported to be used in direct hair dye formulations. While the safety of individual hair dye ingredients is not addressed in epidemiology studies that seek to determine links, if any, between hair dye use and disease, such studies do provide broad information. The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer. A detailed summary of the available hair dye epidemiology data is available at https://www.cir-safety.org/cir-findings.

SUMMARY

Basic Blue 99 is reported to function in cosmetics as a hair colorant and a hair conditioning agent. Basic Blue 99 was previously reviewed by the Panel in a safety assessment that was published in 2007. At that time, the Panel concluded that

Basic Blue 99 was safe for use as a hair dye ingredient. In accordance with its Procedures, the Panel evaluates the conclusions of previously-issued reports approximately every 15 years, and it has been at least 15 years since this assessment has been issued. In December 2022, the Panel determined that this safety assessment should be re-opened for re-evaluation due to concerns regarding the variability of the composition of the ingredient. The SCCS reported that the purity of Basic Blue 99 (as cation) was 58.0 - 70.0%, and that several isomeric forms and a mixture of up to 40 chemical analogues may comprise a single bath of Basic Blue 99. There are no restrictions for use of Basic Blue 99 by the European Union.

According to 2022 VCRP survey data, Basic Blue 99 has 38 reported uses; non-hair dye uses have been reported, including 1 use in nail polish and enamel and 6 uses in non-coloring hair products. The results of the concentration of use survey provided by the Council in 2022 indicated this ingredient is used in hair dyes and colors at a maximum concentration of 0.2%. When the original safety assessment was published in 2004, Basic Blue 99 was reported to have 51 uses in hair coloring products. In 2002, the maximum concentration of use for Basic Blue 99 in hair coloring products was reported to be 2% in hair tints.

The utility of the EHE 3D model was studied using Basic Blue 99 (> 63% pure). In this study, Basic Blue 99 had more pronounced necrotic death in monolayer cultures, while apoptosis was observed in a 3D environment. Based on the data generated from the EHE 3D model, the authors stated a decrease in the keratinocytes viability was expected after human dermal exposure to Basic Blue 99, which could result in dermal irritation. (It should be noted that this study was published before the OECD TG on reconstructed human epidermis 3D models was finalized, and it does not meet the OECD TG requirements.) Case reports concerning reactions to Basic Blue 99 in hair dye formulations have been described in published literature.

The Panel determined that the available hair dye epidemiology data do not provide sufficient evidence for a causal relationship between personal hair dye use and cancer.

Method of manufacturing data and carcinogenicity studies on Basic Blue 99 were not included in the original safety assessment and were not found in an updated search of the published literature, and unpublished data were not submitted.

PREVIOUS DISCUSSION

The Panel was initially concerned with evidence of dermal sensitization.[2] The Panel accounted for the differing results in two LLNA studies as an effect of the type of vehicle used (DMSO or twice-distilled water). Moreover, a repeated-insult patch test using 54 volunteers in an exaggerated exposure (nine 1-h induction exposures followed by a 1-h rechallenge up to 15 d later) did not cause adverse responses. These data, coupled with the negative results in guinea pigs, led the Panel to conclude that there was not a significant risk of skin sensitization. The Panel expects that individuals will perform the preliminary testing on or by individuals, as described in product labeling, using an open patch test that is evaluated at 48 h after application of the test material, as advised in product labeling. Users, therefore, would be able to determine their individual reactions to hair dye products containing Basic Blue 99.

The Panel stated that use of direct hair dyes, although not the focus in all epidemiology studies, appears to have little evidence of an association with cancer and other adverse events. The low dermal absorption of Basic Blue 99, the weak results in the Ames assays, and the negative mammalian genotoxicity led the Panel to conclude that there was little carcinogenic risk of this direct hair dye.

DISCUSSION

To be determined.

CONCLUSION

To be determined.

TABLES

Table 1. Chemical properties

Property	Value	Reference
Physical Form	Blue powder	2
Odor	Odorless	2
Formula Weight (g/mol)	451.8	2,3
Density (g/ml @ 20 ºC)	2.19	13
Vapor pressure (mmHg)	1.965×10^{-19} (estimated)	13
Melting Point (ºC)	300 - 320	2
Boiling Point (ºC)	724.99 (estimated)	13
Water Solubility (g/l)	1.37 (estimated) 10-100 (20 ºC)	13 3
Other Solubility (g/l @ 20 ºC)	Ethanol: 1-10 DMSO: 1-10	3
log K_{ow}	-0.88 (estimated) 1.88 (estimated)	13 3
UV Absorption (λ; nm)	270, 577, 619	2

Table 2. 2022 and historical frequency and concentration of use according to likely duration and exposure and by product category.

	# of Uses		*Max Conc of Use (%)*	
	Basic Blue99			
	2022[4]	**2002[2]**	**2022[5]**	**2002[2]**
Totals	**38**	**51**	**0.2**	**0.004-2**
summarized by likely duration and exposure*				
Duration of Use				
Leave-On	*1*	*NR*	*NR*	*NR*
Rinse-Off	*37*	*51*	*0.2*	*0.004-2*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	NR	NR
Incidental Inhalation-Powder	NR	NR	NR	NR
Dermal Contact	NR	NR	NR	NR
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	6	NR	NR	NR
Hair-Coloring	31	51	0.2	0.004-2
Nail	1	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR
as reported by product category				
Hair Preparations (non-coloring)				
Hair Conditioner	2	NR	NR	NR
Shampoos (non-coloring)	1	NR	NR	NR
Other Hair Preparations	3	NR	NR	NR
Hair Coloring Preparations				
Hair Dyes and Colors (all types requiring caution statements and patch tests)	6	18	0.2	0.3-0.4
Hair Tints	NR	25	NR	2
Hair Rinses (coloring)	10	NR	NR	0.2-1
Hair Shampoos (coloring)	7	8	NR	0.125
Hair Lighteners with Color	1	NR	NR	NR
Other Hair Coloring Preparation	7	NR	NR	0.004-0.4
Manicuring Preparations (Nail)				
Nail Polish and Enamel	1	NR	NR	NR

NR – not reported

*likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

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International Journal of Toxicology, 26(Suppl. 2):51–63, 2007

ISSN: 1091-5818 print / 1092-874X online
DOI: 10.1080/10915810701351202

Final Report on the Safety Assessment of Basic Blue 99[1]

Basic Blue 99 is a direct, nonoxidative hair colorant used in temporary and semipermanent hair dyes. According to current reported usage data, Basic Blue 99 is used at concentrations from 0.004% to 2% and the most often reported use is in hair tints. Hair dyes containing Basic Blue 99, as "coal tar" hair dye products, are exempt from the principal adulteration provision and from the color additive provision 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. Preliminary testing on or by individuals should be done using an open patch test that is evaluated at 48 h after application of the test material. Users, therefore, would be able to determine their individual reactions to hair dye products containing Basic Blue 99. Basic Blue 99 dye is approximately 60% to 63% dye, whereas the remainder of the mixture is composed of sugar (∼25.7%), volatile matter/water crystallization (∼1.8%), and inorganic salts (bringing the mixture to 100%). The dermal absorption of Basic Blue 99 is low in both rats and humans. The LD_{50} values of Basic Blue 99 in mice and rats were 2.7 g/kg and between 1.0 g/kg and greater than 2.0 g/kg, respectively. Mice and rats orally administered Basic Blue 99 for 90 days did not show any indications of cumulative toxicity. Discoloration of organs involved in the elimination of Basic Blue 99 from the animals was noted in both test species. In rabbits, Basic Blue 99 did not cause ocular irritation, but some discoloration was noted. Basic Blue 99 caused minimal dermal irritation in rabbits. Sensitization occurred in animals exposed to Basic Blue 99 in a DMSO vehicle, but not in a water vehicle in guinea pigs and mice. Basic Blue 99 administered by gavage did not cause developmental toxicity in rats. Basic Blue 99 was a weak mutagen with and without metabolic activation in the Ames test, producing both reverse and frameshift mutations, but did not induce mutations in *Escherichia coli* or in any mammalian cells tested. In a modified repeated-insult patch test (RIPT), no volunteers had any reaction to Basic Blue 99 after a 1-h occlusive challenge. Case reports have documented positive patch test results to 1% Basic Blue 99 in three patients. A current review of the hair dye epidemiology literature identified that use of direct hair dyes, although not the focus in all investigations, appears to have little evidence of an association with cancer or other adverse events. The Panel recognizes that hair dye epidemiology studies do not address the safety of individual hair dyes. Based on the available safety test data on Basic Blue 99, however, the Panel determined that this ingredient would not likely have carcinogenic potential as used in hair dyes. The Cosmetic Ingredient Review Expert Panel concluded that Basic Blue 99 is safe as a hair dye ingredient in the practice of use and concentration as described in this safety assessment.

Received 30 November 2006; accepted 5 March 2007.

[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. Address correspondence to F. Alan Andersen, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036, USA.

INTRODUCTION

Basic Blue 99 is a direct, nonoxidative hair colorant used in temporary and semipermanent hair dyes. This review presents information relevant to the safety of Basic Blue 99 as a direct hair dye cosmetic ingredient as considered by the Cosmetic Ingredient Review (CIR) Expert Panel.

CHEMISTRY

Definition and Structure

As described in the *International Cosmetic Ingredient Dictionary and Handbook* (Gottschalck and McEwen 2004) Basic Blue 99 (CAS no. 68123-13-7) is the naphthoquinoneimine color that conforms to the empirical formula: $C_{19}H_{20}BrN_4O_2 \cdot Cl$ and the structural formula shown in Figure 1.

As reported by Steiling (2002), Basic Blue 99 is a commonly used hair dye that consists of a mixture of about 70% chromophores, about 20% sucrose, about 7% inorganic salts ($ZnCl_2$ and NaCl), and about 4% water. The chromosphore component is predominantly (approximately two-thirds) three isomers of bromo-4,8-diamino-6-(3-trimethylamino)-phenylamino-1,5-naphthochinon, in which the position of the bromo group occupies the 2, 3, 6, or 7 position.

In another description, de Groot and Weyland (1990) stated that Basic Blue 99 is a mixture of two quaternary ammonium compounds that differ in the number of bromine atoms and the position of the trimethylanilinium group.

Physical and Chemical Properties

Table 1 presents the available physical and chemical properties of Basic Blue 99, along with a list of synonyms and trade names.

Analytical Methods

Basic Blue 99 may be identified by its absorption spectra in the ultraviolet (UV) and infrared (IR) regions. The UV spectrum of Basic Blue 99 is depicted in Figure 2, with peaks at 270, 577, and 619 nm (Henkel 1992).

Figure 3 gives the IR spectra of Basic Blue 99 (Keystone Aniline Corporation 1999).

Impurities

According to the Keystone Aniline Corporation (1999), Basic Blue 99 must be at least 63% pure Basic Blue 99 in the color mixture and have no more than 100 ppm of iron.

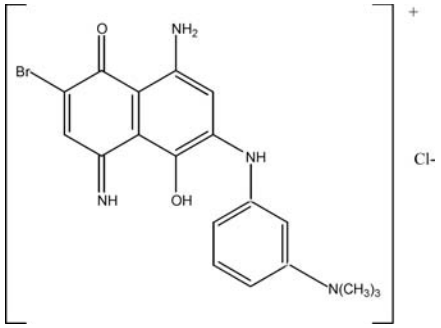

FIGURE 1
Structure for the naphthoquinoneimine color Basic Blue 99 (Gottschalck and McEwen 2004).

Another supplier of Basic Blue 99 (Henkel 1992) has a specification of 60.7% dye content, 25.7% sugar content, 11.8% inorganic salts, and 1.8 % volatile matter/water crystallization. The majority of the additional contents of hair dye are understood as being part of the color and not undesirable chemical impurities.

USE

Cosmetic

Basic Blue 99 is used as a color additive in the following product categories: hair-coloring preparations (miscellaneous), hair dyes and colors [all types requiring statements and patch tests (as discussed below)], hair shampoos (coloring), and hair tints (Gottschalck and McEwen 2004).

Basic Blue 99 has been in production since 1979 and with a yearly production of about 3800 kg worldwide. It is an aminoketone dye that is used in products for dyeing hair, including setting and tonic lotions, and in shampoos. Basic Blue 99 is a semipermanent dye and should last for four to five washes as it penetrates into the cuticle and partially in the cortex of the hair (Wigger-Alberti et al. 1996).

Basic Blue 99 has reported use in five product categories (see Table 2) (FDA 2002). Concentration of use values are no longer reported to the Food and Drug Administration (FDA) by the cosmetic industry (FDA 1992), but industry has reported that current use concentrations range from 0.004% to 2% (CTFA 2002).

The Keystone Aniline Corporation (1999) reported that JarocolColor™ Premixes for formulation in coloring shampoos contain 0.45% to 7.5% Basic Blue 99 (used as a temporary hair dye) and that the actual concentration of Basic Blue 99 in the final cosmetic product would be 0.01% to 0.375%.

Hair dyes containing Basic Blue 99, as "coal tar" hair dye products, are exempt from the principal adulteration provision and from the color additive provision in sections 601 and 706 of the Federal Food, Drug, and Cosmetic Act of 1938 when

TABLE 1
Basic Blue 99: Physical/chemical properties and synonyms

Molecular formula	$C_{19}H_{20}BrN_4O_2 \cdot Cl$	Gottschalck and McEwen 2004; US EPA 2002
Synonyms	Benzenaminium, 3-[(4-amino-6-bromo-5,8-dihydro-1-hydroxy-8-imino-5-oxo-2-naphthalenyl)amino]-*N*, *N*, *N*-trimethyl-, chloride; 3-[(4-amino-6-bromo-5,8-dihydro-1-hydroxy-8-imino-5-oxo-2-naphthalenyl)amino]-*N*, *N*, *N*-trimethylbenzenaminium chloride; CI 56069	Gottschalck and McEwen 2004; ChemIDplus 2002
Trade names	Jarocol™ Steel Blue	Gottschalck and McEwen 2004; Keystone Aniline Corporation 1999
	Arianor Steel Blue	Henkel 1992
Molecular weight	451.75	US Environmental Protection Agency (EPA) 2002
	451.73	Keystone Aniline Corporation 1999
Solubility	Soluble in water at 25°C and 60°C Slightly soluble in isopropyl alcohol at 60°C Insoluble in isopropyl alcohol at 25°C	Keystone Aniline Corporation 1999
	Soluble in ethanol	Henkel 1992
Description	Blue powder	Keystone Aniline Corporation 1999
	Dark blue powder	Henkel 1992
Odor	Odorless	Henkel 1992
Melting point	300–320°C	Henkel 1992
UV max.	270, 577, 619 nm	Henkel 1992

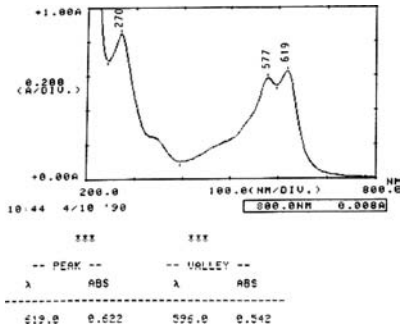

FIGURE 2
Ultraviolet radiation spectrum for Basic Blue 99 (Henkel 1992).

the label bears a caution statement and "patch test" instructions for determining whether the product causes skin irritation (FDA 1979). 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.

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 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 of 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). Basically, 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 at 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 cosmetics 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 cosmetics 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.

Accordingly, preliminary testing on or by individuals should be done using an open patch test that is evaluated at 48 h after application of the test material.

Basic Blue 99 is listed in section 1 of the "First Update of the Inventory of Ingredients Employed in Cosmetic Products" with a stated function as a hair dyeing ingredient with no listed restrictions (European Union On-Line 2000).

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

Wolfram (1984) studied the skin permeation of Basic Blue 99 in test animals and human volunteers. Three Sprague-Dawley rats were treated topically with 200 μl of an aqueous Basic Blue 99 (^{14}C labeled) hair setting solution to assess its skin permeation potential. Approximately 31.3 μg Basic Blue 99/cm^2 skin was applied at a volume of 0.2 ml over a 1.5 × 1.5-cm area on intact, clipped dorsal aspect of the thorax. One hour later each rat was fitted with a collar to prevent licking the area of application. Urine and feces were analyzed 24 h after application. Levels of ^{14}C were low in the urine (< 0.02%) and feces (<0.07%) of two rats. The third rat excreted more than 5.05 % of ^{14}C dose in urine, but only 0.12% in feces. The study concluded there is

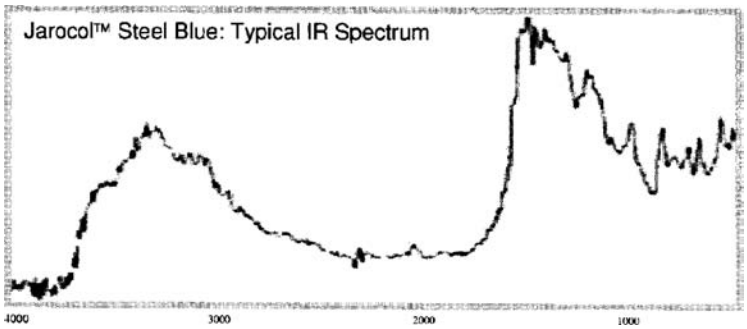

FIGURE 3
IR absorption spectrum for Jarocol brand of Basic Blue 99 (Keystone Aniline Corporation 1999).

TABLE 2
Basic Blue 99: Product categories and concentration of use

Product category (Total number of products in each category) (FDA 2002)	Ingredient uses (FDA 2002)	Use concentrations (CTFA 2002) (%)
Hair-coloring products		
Dyes and colors (1690)	18	0.3–0.4
Tints (49)	25	2
Rinses (20)	—	0.2–1
Shampoos (32)	8	0.125%[a]
Other hair-coloring preparations (55)	—	0.004–0.4
Total	51	0.004–2

[a]Noveon, Inc. 1999.

very low percutaneous absorption of Basic Blue 99 in rats.

Two female volunteers were treated topically with a hair setting solution containing Basic Blue 99. ^{14}C-labeled Basic Blue 99 was added to a setting lotion (40% aqueous solution containing 0.1% of other Arianor dyes) and applied with rollers to the hair of the volunteers. The hair was dried for 10 min under a hair dryer, the rollers were removed and the hair was combed out. The volunteers shampooed their hair 36 h later. The percutaneous absorption was measured in urine over the next 8 days. Levels of ^{14}C in the urine were low in both volunteers (0.037% and 0.027%). The authors concluded that the penetration would be less than 0.1% of the applied dose (Wolfram 1984).

Parish (1988) conducted studies of Basic Blue 99 skin penetration as a function of the cosmetic vehicle. Six Wistar rats (three males, three females) were treated topically with 100 μl of an aqueous 1% Basic Blue 99 (^{14}C labeled) solution. Application was made over 10 cm^2 of intact, clipped dorsal skin and was occluded for 48 h. Most of the recovered ^{14}C label was associated with the treated area of the skin or the patch; moreover, levels of ^{14}C were barely detectable in the urine, expired air, feces, blood, and carcasses. The total amount of material that penetrated the skin was 0.14% and 0.06% in male and female rats, respectively. The vehicle did have a small effect on the skin penetration of Basic Blue 99; the highest absorption was seen in the anionic shampoo, then in the cationic and nonionic bases, and with the lowest penetration seen with the aqueous solution. However, even the highest absorption was very low and the author deemed it insignificant.

This author also treated four female Wistar rats topically with 100 μl of an aqueous 1% Basic Blue 99(^{14}C labeled) solution in a 50% aqueous anionic shampoo base. The chemical application was over 10 cm^2 on intact, clipped dorsal skin. In one group an occlusive patch was applied for 48 h, and in the other group the treatment was rinsed with distilled water after 5 min and a nonocclusive patch was applied. In both groups most of the recovered ^{14}C label was on the skin or the patch. Small amounts of ^{14}C were detected in the urine (1.16%), feces (0.53%), and carcass (0.03%). Only 1.72% of the applied dye penetrated the skin under occlusive conditions. Penetration was reduced in animals that were rinsed after application; ^{14}C was detected in the urine and feces (0.02%), blood and carcass (<0.001%), and only 2.9% of the applied material remained on the skin surface at 48 h after treatment.

This author also treated four female Wistar rats topically with 100 μl of an aqueous 1% Basic Blue 99 (^{14}C labeled) solution in a 25% aqueous cationic hair conditioner. Prior to application, the treated area of skin was prewashed with an anionic shampoo to simulate consumer use. Application was as above. In both groups most of the recovered ^{14}C label was on the skin or the patch. Small amounts of ^{14}C were detected in the urine, feces, and carcass (0.47%), with <0.001% in blood. Penetration was reduced in animals that were rinsed after application; ^{14}C was detected in the urine and feces (0.04%), blood and carcass (<0.001%), and 11.1% of the applied material remained on the skin surface at 48 h after treatment. Most of the radioactivity was recovered in the rinsing (74%) and 3.8% was on the patch.

This author also treated four female Wistar rats topically with 100 mg of 0.5% Basic Blue 99(^{14}C labeled) in nonionic/cationic shampoo base. Application was as given above. In both groups most of the recovered ^{14}C label was on the skin or the patch. Small amounts of ^{14}C were detected in the urine, feces, and carcass (0.5%) with <0.001% in blood. Penetration was reduced in animals that were rinsed after application; ^{14}C was detected in the urine (0.01%), feces (0.02%), blood and carcass (<0.001%), and 1.68% of the applied material remained on the skin surface at 48 h after treatment. Most of the radioactivity was recovered in the rinsing (99%) and 1.3% was on the patch (Parish 1988).

In an oral study, Parish (1988) gave six Wistar rats (three males, three females) a single oral dosage of ^{14}C-labeled Basic Blue 99 (1.0 ml of an aqueous 0.1% solution). The amount of radioactivity was determined in urine, feces, expired air, and in the carcass at the end of the 48-h observation period. Most of the ^{14}C was recovered in the feces within the first 24 h. The urine contained 0.68% and 0.5% of the dose from male and female rats, respectively. Levels of ^{14}C were barely detectable in expired

air and were very low in the blood and carcass (<0.001%). The study concluded Basic Blue 99 is poorly absorbed from the intestinal tract.

Wolfram (1984) gave two male Sprague-Dawley rats a single intraperitoneal dose (1 ml) of ^{14}C-labeled Basic Blue 99 in water (0.912 μCi/ml). Another two rats were administered Basic Blue 99 orally. The amount of radioactivity was determined in urine and feces until the end of the 48-h observation period. Most of the ^{14}C was recovered in the feces in both study groups within the first 24 h. The urine contained 10.1% or 3.3% of the dose after intraperitoneal administration and 2.8% or 3.1% after oral administration. The author concluded Basic Blue 99 is poorly absorbed.

Antimicrobial Activity

Basic Blue 99 (0.1%) killed *Streptococcus sanguis* bacteria (creating a growth-free zone) after exposure to a He/Ne laser for 10 and 60 s. Basic Blue 99 (0.01%) did not produce a growth-free zone after exposure to a He/Ne laser for 10 and 60 s (Dobson and Wilson 1992).

ANIMAL TOXICOLOGY

Acute Oral Toxicity

Kynoch and Lloyd (1977) studied the acute oral toxicity of Basic Blue 99 using groups CFY rats. Basic Blue 99 was prepared as a 10% w/v suspension in aqueous methylcellulose (1%) and administered via oral intubation at a dosage volume of 1.0 to 40 ml/kg body weight. Groups of four rats (two males, two females weighing 84 to107 g) were administered 0.1 to 4.0 g/kg body weight. Controls received vehicle alone. Animals were observed for 14 days. Immediately following Basic Blue 99 administration, piloerection and hunched posture were observed in all rats. These signs were accompanied by lethargy, pallor of the extremities and ptosis in female rats at 1.0 g/kg and all rats at 2.0 and 4.0 g/kg, by increased salivation in rats at 2.0 g/kg, and by diuresis and fine body tremors in rats at 4.0 g/kg. Blue staining of the urine and saliva was noted in rats of the 2.0 and 4.0 g/kg groups. There were no deaths of male rats after a single oral dose of Basic Blue 99 up to 1.0 g/kg, but one female died at this dose. One rat of each sex died in the 2.0 g/kg group and all rats in the 4.0 g/kg died within 1 week of dosing. Necropsy revealed slight injection of the blood vessels of the abdominal viscera, pallor of the spleen and liver, and discoloration of the kidneys. The LD_{50} was reported to be between 1.0 and 2.0 g/kg.

Kynoch (1986) conducted a follow-up acute oral toxicity study of Basic Blue 99 using groups of 10 Sprague-Dawley rats (5 per sex; 134 to 152 g). Basic Blue 99 was prepared as a 20% suspension in distilled water (w/v) and administered at a dosage volume of 10 ml/kg body weight. Rats were given a single oral dose of 2.0 g/kg body weight Basic Blue 99 using a syringe and a plastic catheter. Animals were observed for 14 days and killed on day 15. Immediately following Basic Blue 99 administration, piloerection, hunched posture, abnormal gait, and increased salivation were observed in all rats, but recovery was complete by day 3. There were no deaths. The LD_{50} was stated as >2.0 g/kg.

Henkel (1990a) assessed the acute oral toxicity of Basic Blue 99 using groups of 10 male CF1 mice (20 to 22 g). Basic Blue 99 was applied once by gavage at six dosages between 1.58 and 5.01 g/kg body weight at a dosage volume of 20 ml/kg body weight. Animals were observed for 7 days. Immediately following Basic Blue 99 administration decreased activity, increased breathing, and tremors were noted at doses of 1.58 g/kg and greater. There were no deaths at the lowest dose of 1.58 g/kg. The LD_{50} was reported to be 2.70 g/kg.

Subchronic Oral Toxicity

Wella Aktiengesellschaft (1978) investigated the effects of Basic Blue 99 on CF1 female mice (average 21 g) in a 90-day oral toxicity study. Ten female mice per group were fed Basic Blue 99 daily in the diet at 125, 250, and 500 mg/kg. Twenty control mice received only diet.

All mice survived the duration of the study. There were no indications of cumulative toxicity in hematological, biochemical, and urological interim and final examinations. Stained urine was observed in all dosed mice. No differences in behavior or organ weight were found between dosed and control mice. No direct correlation was found between the applied dose and body weight gain; however, at the end of the study the mean body weight of treated groups was generally lower than the control group. Discoloration of stomach and intestines were observed grossly. Histologically, histiocytic cell infiltration, presence of fat, and hemosiderosis were found in the liver of the dosed animals, but not in control animals. Hemosiderosis in the spleens of the treated animals was comparable to the control animals. The findings were not considered dose related. The authors concluded that none of the Basic Blue 99 doses tested led to cumulative toxic effects (Wella Aktiengesellschaft 1978).

Henkel (1986) investigated the effects of Basic Blue 99 on Sprague-Dawley rats (males 64 to 80 g; females 62 to 79 g) in a 90-day oral toxicity study. Male and female rats (10 animals per sex in each dose group) received 0 (control), 20, 60, and 180 mg/kg Basic Blue 99 daily by oral gavage. The highest dose was increased to 360 mg/kg after 8 weeks. The controls were treated simultaneously with the aqueous vehicle at volumes of 10 ml/kg body weight.

All rats survived the treatment period without signs of intoxication. Body weight gain was comparable to controls except in high-dose males, which showed decreased body weight gain; however, the difference was not statistically significant. Urine was stained at all doses. There was no indication of cumulative toxicity in hematological, biochemical, and urological interim and final examinations. Discoloration of the stomach and adrenals were observed grossly at the highest dose, and discoloration of the forestomach was observed at the middle dose.

Histologically, singular foreign pigment granula were observed in the villi of the small intestine at all doses. As these pigmentations were found only in organs involved in the elimination of Basic Blue 99, these findings were not considered relevant as a possible induction of toxic effects. The study concluded none of the doses led to cumulative toxicity and that the no effect level for Basic Blue 99 was between 180 to 360 mg/kg body weight daily (Henkel 1986).

Ocular Irritation

Leuschner (1967a) evaluated the acute eye irritation of Basic Blue 99 using three albino New Zealand rabbits. A solution of 0.5% Basic Blue 99 (0.1 ml) was instilled into the conjunctinal sac of the left eye and the right eye received 0.1 ml of vehicle alone (saline). Eye irritation was read at 30 and 60 min and 1 and 2 days post instillation. The conjunctivae of the Basic Blue 99 treated eye were discolored, but no effects on the cornea or iris were observed in any animal. The study concluded that the mucous membrane injury threshold concentration for the rabbit eye is greater than 0.5% Basic Blue 99.

Dermal Irritation

Leuschner (1967b) assessed the dermal irritation potential of Basic Blue 99 using six albino New Zealand rabbits. Basic Blue 99 (0.5 g per square inch) was applied undiluted to either the shorn intact (three animals per sex) or abraded skin (three animals per sex) on the back of animals. The patch was affixed for 24 h. No observable reactions to Basic Blue 99 were noted over the 14-day observation period.

Kynoch and Liggett (1977) assessed the dermal irritation potential of Basic Blue 99 using six albino rabbits. Basic Blue 99 (0.5 g) was dampened with 0.5 ml distilled water to a 1-square-inch area on either the shorn intact or abraded skin on the back of animals. The patch was affixed for 24 h. Very slight erythema and edema were observed in the intact and abraded sites of one animal at the 24-h reading only. The primary irritation index was calculated to be 0.2 and Basic Blue 99 was considered "mildly irritating" to rabbit skin.

Dermal Sensitization

Kynoch and Elliott (1977) conducted a guinea pig maximization test using 10 female albino Hartley/Dunkin guinea pigs to assess the sensitization potential of Basic Blue 99. For induction, a 4 × 6-cm area of dorsal skin on the scapular region was clipped free of hair and three pairs of injections were made simultaneously: (1) Freund's complete adjuvant (FCA) mixed 50:50 in water (v/v); (2) Basic Blue 99 as a 0.1% (w/v) solution in water; (3) Basic Blue 99 as above in water mixed 50:50 with FCA (v/v). One week after the injections, a volume of 0.40 ml of 75% Basic Blue 99 solution was applied onto a 3 × 6-cm patch and held in place for 48 h. Two weeks after the induction period, animals were challenged topically with 0.1 ml of 25% Basic Blue 99 applied to a patch and held in place on the flank of each animal for 24 h. Skin reactions were read at 24, 48, and 72 h after challenging. Basic Blue 99 did not produce any evidence of delayed contact hypersensitivity.

RCC Ltd. (2001a) performed a local lymph node assay (LLNA) to assess the contact allergenic potential of Basic Blue 99 when administered to the dorsum of both ear lobes of CBA/J female mice. There were three treated groups (four mice per group) receiving 1%, 5%, or 25% Basic Blue 99 in bidistilled water for 3 consecutive days. A control group of four mice received the vehicle only and the positive control group received either 5%, 10%, or 25% α-hexylcinnamaldehyde. Five days after the first topical application, the mice were injected intravenously with ^{3}H-thymidine. Five hours later, mice were killed and the auricular lymph nodes were removed. The lymph node cells were incubated with trichloroacetic acid overnight and the incorporation of ^{3}H-thymidine was determined using a β-scintillation counter. A response was considered positive in the LLNA assay if the exposure resulted in a threefold or greater increase in incorporation of ^{3}H-thymidine as compared to the solvent control.

All treated animals survived the study period and no clinical signs were observed that related to Basic Blue 99 exposure. Mice exposed to 1%, 5%, and 25% Basic Blue 99 showed an increased incorporation of ^{3}H-thymidine at 0.7-, 1.1-, and 1.1-fold, respectively as compared to the solvent control. The positive-control mice exposed to 5%, 10%, and 25% α-hexylcinnamaldehyde had an increased incorporation of ^{3}H-thymidine at 2.4-, 3.7-, and 7.0-fold, respectively as compared to the solvent control. Basic Blue 99 was considered a nonsensitizer in this study (RCC Ltd. 2001a).

Calvert Preclinical Services, Inc. (2002) reported a LLNA study of Basic Blue 99. CBA/J female mice (five per dose group) were treated on the dorsal surface of both ears once daily for 3 days with 0.25%, 0.50%, 1.0%, or 2.0% (w/v) of Basic Blue 99 at a volume of 25 μl/ear. Positive control mice received *p*-phenylenediamine (PPD) and negative-control mice received the vehicle, DMSO. Irritation and body weights were recorded. On day 6, mice were injected intravenously with 20 μCi of ^{3}H-thymidine. Five hours later, mice were killed and the auricular lymph nodes were removed. The lymph node cells were precipitated with 5% trichloroacetic acid and the incorporation of ^{3}H-thymidine was determined using a β-scintillation counter.

No irritation was noted in any mice as a result of Basic Blue 99 treatment. The positive control (PPD) resulted in test/control ratios of greater than 3 at 0.25%, 0.50%, 1.0%, and 2.0% (4.53, 10.06, 9.99, and 15.74, respectively), indicating a positive response. Basic Blue 99 at 0.5%, 1.0%, and 2.0% gave responses statistically significantly greater than the vehicle control, but not test/control ratios greater than 3. A positive response was also observed in the 0.25% group, but the difference was not statistically significant as compared to the vehicle control. The mean body weights and mean changes in body weight of treated mice were not significantly different than those of vehicle-control mice. The authors concluded that the assay results indicated

Basic Blue 99 may induce a hypersensitivity response (Calvert Preclinical Services, Inc. 2002).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Henkel (1990b) evaluated the effects of Basic Blue 99 (50 mg/kg/day) administered by gavage to 29 Pregnant Sprague-Dawley CD rats on the 6th to the 15th gestation days. Controls animals were dosed with distilled water vehicle. On day 20 the rats were killed and cesarean sections were performed. The number of implantation sites, resorptions, living fetuses, and the number of corpora lutea were counted in each dam. The weight of the placenta, uterus, fetuses, dams, body weight gain, and sex of the fetuses were recorded. One-third of the litter was examined for soft tissue anomalies and the remaining fetuses were examined for skeletal anomalies.

No dams died or showed cumulative toxicity effect from the applied dose of 50 mg/kg Basic Blue 99. Test animals had no differences in mean body weight gain in the course of gestation as compared to controls. There were no treatment related effects. The authors concluded that Basic Blue 99 at 50 mg/kg did not cause embryotoxic or teratogenic effects under the test conditions (Henkel 1990b).

GENOTOXICITY

Bacterial Assays

The Battelle Institut (1975) determined the mutagenic potential of Basic Blue 99 using *Escherichia coli* strain 343/113 without metabolic activation. Basic Blue 99 was tested at the concentrations of 1, 10, and 100 μg/ml and a volume of 0.1 ml in a dark place at 37°C for 2 h. Cells were spread over four selected media and incubated for 20 to 72 h. Cells were counted and analyzed for reverse mutations of arg^- to arg^+ and nad^- to nad^+, forward mutations of 5-methyl-dl-trytophan-sensitivity to MT resistance, and forward and reverse mutations of gal R^s_{18} to gal^+. The mutant colonies were counted and compared with controls. Basic Blue 99 was not mutagenic at the concentrations tested.

Hossack et al. (1977) assessed the mutagenicity of Basic Blue 99 (1 to 1000 μg/plate) in the Ames test using *Salmonella typhimurium* strains TA1535, TA1537, and TA1538 with and without metabolic activation. β-Naphthylamine, neutral red, and 2-acetylaminofluorene were used as positive controls. Basic Blue 99 induced reverse mutations in the absence of S9 in strain TA1537 and in the presence of S9 in strain TA1538. Basic Blue 99 did not induce mutagenic activity in strain TA1535.

Wallat (1985) studied Basic Blue 99 (4 to 2500 μg/plate) in the Ames test using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 with and without metabolic activation. Sodium azide (TA100, TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA1538, TA98) were used as positive controls with and without metabolic activation. 2-Aminoanthracene was used as a positive control for all strains. Basic Blue 99 induced reverse mutations in the absence of S9 in strain TA100 and in the presence of S9 in strains TA98, TA100, TA1537, and TA1538. Basic Blue 99 was not mutagenic in strain TA1535.

Cytotest Cell Research GMBH (2000) investigated the mutagenicity of Basic Blue 99 using *S. typhimurium* with and without metabolic activation (S9). *S. typhimurium* strains TA98 and TA100 were only tested without metabolic activation at 3, 10, 33, 100, 333, and 1000 μg/plate. *S. typhimurium* strains TA1535, TA1537, and TA102 were tested at 3, 10, 33, 100, 333, and 666 μg/plate without metabolic activation and at 33, 100, 333, 666, 1000, and 2500 μg/plate with metabolic activation. Concurrent untreated and solvent controls were performed. The positive controls without metabolic activation were sodium azide, 4-nitro-*o*-phenylenediamine, and methyl methane sulfonate. The positive control with metabolic activation was 2-aminoanthracene.

Irregular background growth was observed at 2500 μg Basic Blue 99/plate in strains TA1537 and TA100 with S9 and at 333 μg/plate in strains TA98 and TA100 and at 666 μg/plate in strains 1535 without S9 and TA1537 with and without S9. There was an increase in revertant colony numbers after treatment with Basic Blue 99 in strains TA102 and TA100 with metabolic activation and in strains TA1537 and TA98 with and without metabolic activation. Positive controls showed the expected increase in revertant colonies. The authors concluded that Basic Blue 99 induced gene mutations by base pair changes in TA100 and TA102 with metabolic activation and frameshift mutations in strains TA1537 and TA98 with and without metabolic activation (Cytotest Cell Research GMBH 2000).

Mammalian Cell Assays

Banduhn (1987) assessed the mutagenicity of Basic Blue 99 in a micronucleus assay in bone marrow cells from male and female CFW 1 mice (21 to 33 g). Basic Blue 99 (in aqueous 0.9% NaCl solution) was administered by gavage to 12 mice (6 mice per sex per test group) at a dose of 1500 mg/kg body weight. Endoxan was the positive control and the vehicle was the negative control. The incidence of micronucleated erythrocytes was evaluated at 24, 48, and 72 h by preparing bone marrow smears from both femurs from each animal. Of the treated mice, three of six and four of six male mice died in the 24- and 72-h observation groups. The remaining animals from the 72-h group were combined with the 24-h group, and the 72-h group was abandoned. There was no indication of mutagenic activity of Basic Blue 99 as determined by bone marrow examination of the remaining groups and no indication of a delayed cell proliferation. No additional tests were performed to determine the micronucleus rate in male mice in the 72-h group. The study concluded Basic Blue 99 did not show any evidence of mutagenic potential under these test conditions.

Timm (1988) evaluated the ability of Basic Blue 99 to induce DNA repair in rat hepatocytes using an unscheduled DNA synthesis (UDS) assay. In two replicate studies, Basic Blue 99 was tested at 1.00, 3.33, 10.00, 33.33, and 100.00 μg/ml and incubated for 3 h. UDS was determined using liquid scintillation

counting. The positive control, 2-acetylaminofluorene, produced significant repair synthesis. A reduction in the incorporation of radioactivity occurred at 33.33 and 100.00 μg/ml in experiment I, which indicated weak toxicity. Concentrations higher than 100.00 μg/ml were very toxic. The incorporation of thymidine into rat hepatocytes was not dose related in either experiment. The study concluded that, due to an insignificant difference in UDS between Basic Blue 99 and negative controls, Basic Blue 99 did not induce DNA repair synthesis.

Michalke (1991) assessed the potential for Basic Blue 99 to induce structural chromosome aberrations in V79 cells of the male Chinese hamster in vitro with and without metabolic activation. V79 cells were exposed to Basic Blue 99 (in dimethylsulfoxide vehicle) for 24 hours at 0, 0.1, 0.3, 0.5, 1.0, 2.5, 3.0, 5.0, or 10.0 μg/ml without S9 and for 2 h at 3, 10, 25, 30, 50, 100, 125, 150, or 250 μg/ml with S9. Negative, solvent, and positive controls were used. With and without S9 Basic Blue 99 did not induce an increase of thioguanine-resistant clone growth in cultured V79 Chinese hamster cells in vitro.

Cytotest Cell Research GMBH (2001) assessed the potential for Basic Blue 99 to induce structural chromosome aberrations in Chinese hamster V79 cells in vitro. V79 cells were exposed to Basic Blue 99 (in deionized water vehicle) for 4 h at 0, 0.5, 0.8, and 1.5 μg/ml without S9 and 15.0, 30.0, and 45.0 μg/ml with S9. The chromosomes were prepared 18 h after the start of treatment with Basic Blue 99. Negative, solvent, and positive controls were used.

At the highest concentration, with and without metabolic activation, there were reduced cell numbers after 4 h of treatment. Without metabolic activation, there were significant increases in the number of cells with structural chromosomal aberrations after treatment with 0.5, 0.8, and 1.5 μg/ml Basic Blue 99 (with increases of 4.0%, 14.5%, and 9.5%, respectively). With metabolic activation, there were a significant increase in the number of cells with structural chromosomal aberrations after treatment with 45 μg/ml Basic Blue 99 (with an increase of 12.0%). No increase in the frequencies of polyploid metaphases were found after treatment as compared to controls. Appropriate positive controls induced a statistically significant increase in chromosomal aberrations, whereas negative and solvent controls did not induce a statistically significant increase in chromosomal aberrations. Basic Blue 99 was considered to be clastogenic with or without metabolic activation (Cytotest Cell Research GMBH 2001) .

Animal Assays

RCC Ltd. (2001b) assessed the mutagenicity of Basic Blue 99 in a micronucleus assay in bone marrow cells of the mouse. Basic Blue 99 was administered intraperitoneally to mice at a volume of 10 ml/kg body weight (bw). Basic Blue 99 was administered at 0.2, 1.0, and 5.0 mg/kg bw and bone marrow cells were collected for analysis 24 hours post-administration; and at 5.0 mg/kg bw, with bone marrow cells collected for 48 h post administration. Ten animals (5 males, 5 females) per test group were evaluated for the occurrence of micronuclei. The ratio between polychromatic and normochromatic erythrocytes (NCEs) was also determined. A 40 mg/kg cyclophosphamide dose was used as a positive control.

One male in the highest dose group died in the 48-h preparation. Basic Blue 99 did not substantially increase the number of NCEs as compared to the mean values of NCEs of the vehicle controls. In comparison to the corresponding vehicle controls, there was no statistically significant or biologically relevant increase in the frequency of detected micronuclei at any preparation interval or dose level as a result of Basic Blue 99 administration. The positive control significantly increased the frequency of induced micronuclei (RCC Ltd. 2001b).

Notox Ltd. (2002) evaluated the ability of Basic Blue 99 to induce DNA repair in male Wistar rat hepatocytes using a UDS assay. Rats were orally dosed with Basic Blue 99 at 250, 500, or 1000 mg/kg body weight at a dosing volume of 10 mg/kg. After 2 to 4 h or 12 to 16 h the hepatocytes were isolated and labeled for approximately 4 h with tritiated thymidine and cultured for 14 to 19 h. Corresponding vehicle controls (saline) served as negative controls, whereas cells treated with single oral doses of dimethylnitrosamine (10 mg/kg bw) or 2-acetylaminofluorene (50 mg/kg) were harvested 2 to 4 h or 12 to 16 h after dosing, respectively.

The results of the negative and positive controls were as expected. At the 2- to 4-h sampling time, there was no positive response to Basic Blue 99 at any dose tested. At the 12- to 16-h sampling time, there was no positive response to Basic Blue 99 at 250 or 1000 mg/kg. However, following oral dosing of male rats with 500 mg/kg Basic Blue 99, the mean net nuclear grain count was increased (2.2 ± 3.3) in one of the three animals. The group average (1.0 ± 1.2) at this dose level was within the range of historical control data. The group average of the percentage of cells in repair was $13.7\% \pm 15.7\%$ (30% and 11% in repair in two animals; the results from the third animal was comparable to the negative control). Since the net nuclear grain count of the group average was still within the range of control data and the number of cells in repair was not higher than or equal to 20%, this increase was considered a chance finding and not biologically significant. The study concluded male Wistar rats showed no induction of DNA repair in hepatocytes isolated 2 to 4 h or 12 to 16 h after dosing with Basic Blue 99 at doses up to 1000 mg/kg and Basic Blue 99 is not genotoxic (Notox Ltd. 2002).

CARCINOGENICITY

No data on the carcinogenicity of Basic Blue 99 were available.

CLINICAL ASSESSMENT OF SAFETY

Dermal Sensitization

A repeated-insult patch test (RIPT) was done to assess the sensitization potential of Basic Blue 99 applied topically.

Fifty-four volunteers (9 males, 45 females, aged 25 to 74 years) completed the study. Modified RIPT methodology was used; there were nine 1-h occlusive induction applications of Basic Blue 99 (0.2 g). Ten to 15 days later, volunteers received a 1-h occlusive challenge application Basic Blue 99 (0.2 g). No reactions were noted in any volunteer tested with Basic Blue 99 (TKL Research, Inc. 2001).

Case Reports

de Groot and Weyland (1990) reported that a 46-year-old woman had applied a colored foam product weekly for 6 months without any side effects, but that 8 h after applying a liquid version of the product unintentionally to the scalp, the patient noticed burning and itching of the scalp and forehead, with redness and swelling of the forehead and upper eyelids. An exudative eruption on the scalp was seen. After 4 days, the patient had significant hair loss. She was first treated 7 weeks post exposure and had thinner hair with localized seborrhoeic-like dermatitis. Five months later most of the hair had regrown.

The patient was patch tested with the European standard series, a cosmetic series, and a hairdressers' series, with negative results. An open test with the product in the elbow fissure resulted in papular dermatitis after 2 days. Later the 37 ingredients (including fragrances) were patch tested. A positive reaction (48 h, ?+; 96 h, ++) was noted to 1% Basic Blue 99 in petrolatum. Seven months later the patient was patch tested using Basic Blue 99 at concentrations of 0.1% in petrolatum (−;+), 1% in petrolatum (?+;++), 0.1% aqueous (?+;+ + +), and 1% aqueous (?+;+++). Twenty-five controls did not react to 1% Basic Blue 99 aqueous and 1% Basic Blue 99 in petrolatum (de Groot and Weyland 1990).

Jagtman (1996) reported that a 71-year-old woman experienced severe itching of the scalp 3 days after application of a hair-setting lotion containing a hair dye. Wheals developed on her trunk and limbs and disappeared after 1 week. After a second application of the lotion, the patient had itching of the scalp and widespread urticaria, which cleared over several weeks (suppressed by an oral antihistamine). The patient was patch tested with the European standard series, a hairdressers' series, and the ingredients in the hair-setting lotion (containing 1% aqueous Basic Blue 99). All tests were negative after 2 and 3 days. Patch tests were performed again and several wheals were present on skin treated with 1% aqueous Basic Blue 99 and the hair-setting lotion hair dye. Scratch tests were performed and reading after 20 min showed +2 reactions to the hair-lotion dye and 1% aqueous Basic Blue 99. No other ingredients produced positive results. Scratch tests were negative in house dust mite, grass pollen, and tree pollen. Scratch tests were negative in 25 patients to 1% aqueous Basic Blue 99. The author concluded the widespread urticaria was suggestive of systemic absorption of Basic Blue 99.

Wigger-Alberti et al. (1996) reported that Basic Blue 99 caused an immediate type allergy in a 67-year-old male hairdresser. Basic Blue 99 is a component of the hair dye "IXI-anthrazit." The patient developed the following symptoms: rhinitis, conjunctivitis, mild coughing, and swelling of the eyelids when the patient came in contact with dyed hair. The skin-prick test was performed with common allergens, cosmetics, "IXI-anthrazit" (undiluted), and the hair dye's components (undiluted). Patch tests were conducted on the back of the patient using several series of materials including 30% "IXI-anthrazit" dissolved in water; test patches were removed after 48 h and the reaction was assessed 24 h later. All skin prick tests were negative, except to IXI-anthrazit, which was positive, and to Basic Blue 99, which was strongly positive. Four people serving as controls were negative to all chemicals in skin-prick tests. No positive patch test reactions were noted in the patient or controls.

HAIR DYE EPIDEMIOLOGY

Hair dyes may be broadly grouped into oxidative (permanent) and direct (semipermanent) hair dyes. The oxidative dyes consist of precursors mixed with developers to produce color, whereas direct hair dyes are a preformed color. Basic Blue 99 is a direct hair dye.

Although 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 and have been considered by the CIR Expert Panel.

In 1993, an International Agency for Research on Cancer (IARC) working group evaluated 78 epidemiology literature citations and concluded that "personal use of hair colourants cannot be evaluated as to its carcinogenicity" and that "occupation as a hairdresser or barber entails exposures that are probably carcinogenic" (IARC 1993). The IARC report did not distinguish between personal use of oxidative/permanent versus direct hair dyes, or distinguish among the multiple chemical exposures in addition to hair dyes to which a hairdresser or barber might be exposed.

In 2003, an updated review of the available epidemiology literature was prepared (Helzlsouer et al. 2003). This review considered 83 literature citations available since the IARC review. The authors found that hair dye exposure assessment ranged from ever/never use to information on type, color, duration and frequency of use. The authors found insufficient evidence to support a causal association between personal hair dye use and a variety of tumors and cancers. The review highlighted well-designed studies with an exposure assessment that included hair dye type, color, and frequency or duration of use, which found associations between personal hair dye use and development of bladder cancer, non-Hodgkin's lymphoma, and multiple myeloma. These findings, however, were not consistently observed across studies.

The CIR Expert Panel did specifically note reports from a case-control study (Gago-Dominguez et al. 2001, 2003), which

did suggest a possible genetically susceptible subgroup, which detoxifies arylamines to a lower degree than the general population. The study authors hypothesized that this subgroup may be at greater risk of bladder cancer from hair dye exposure. Helzlsouer et al. (2003) noted that these results were based on small sample sizes.

Several studies published since the Helzlsouer et al. (2003) review also have been considered. Discussion of the available hair dye epidemiology data is also available at http://www.cir-safety.org/findings.shtml.

Bladder Cancer. Andrew et al. (2004) reported a case-control study of New Hampshire residents whose bladder cancers were entered into a state registry from 1994 to 1998. A follow-up study by Kelsey et al. (2005) examined the links between those bladder cancer cases with an inactivated tumor suppressor gene (TP53) and various exposures. Huncharek and Kupelnick (2005) performed a meta-analysis of six case-control studies and one cohort study. Takkouche et al. (2005) performed a meta-analysis of the Andrew et al. (2004) study and nine other personal use case-control or cohort studies. Ji et al. (2005) reported a cohort occupational study not included in the above meta-analyses. Lin et al. (2006) presented a case-control study of personal permanent hair dye use. Serretta et al. (2006) reported preliminary results from a multicentric study.

Lymphoma and Leukemia. Rauscher et al. (2004) reported a U.S./Canadian case-contol study of adult acute leukemia. Zhang et al. (2004) and Zheng et al. (2004) examined the relationship of hair dye use or diet with non-Hodgkin's lymphoma in a case-control study in Connecticut. Takkouche et al. (2005) reported a meta-analysis of reports of hematopoietic cancers, including those by Rauscher et al. (2004) and Zhang et al. (2004) and 17 other studies. Mester et al. (2005) reviewed 10 epidemiology studies regarding the relationship between occupational exposure in hairdressers and diseases of the malignant lymphoma group. A case-control study in Spain by Benavente et al. (2005) examined the association between lifetime hair dye exposure with various lymphomas, including chronic lymphocytic leukemia.

Other Cancers. Takkouche et al. (2005) included breast cancer and childhood cancers in their meta-analysis. Efird et al. (2005) studied the association between the use of hair-coloring agents the month before or during pregnancy with childhood brain tumors in 1218 cases between 1976 and 1994. Heineman et al. (2005) studied 112 women in Nebraska newly diagnosed with brain cancer (glioma). McCall et al. (2005) reported on the relationship between childhood neuroblastomas and maternal hair dye use in 538 children born between 1992 and 1994 in the U.S. and Canada.

Other Diseases. Park et al. (2005) reported an occupational case-control study of neurodegenerative diseases, including Alzheimer's disease, presenile dementia, and motor neuron disease.

In considering this information, the CIR Expert Panel agreed that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other end points described in the Helzlsouer et al. (2003) review.

The Panel stated that use of direct hair dyes, although not the focus in all investigations, appear to have little evidence of an association with adverse events as reported in epidemiology studies. However, direct hair dyes are a diverse group of chemicals and the determination of safety may hinge on other safety test data.

The Panel recognizes that hair dye epidemiology studies do not address the safety of individual hair dyes, but is concerned that studies have demonstrated an association between use of oxidative/permanent hair dyes and some cancer end points. The Panel, therefore, strongly supports the need to replicate these studies, along with further studies, to examine the possibility of susceptible subpopulations. Additional studies examining bladder cancer, non-Hodgkin's lymphoma, and multiple myeloma and hair dye use are underway and it is the intent of the CIR Expert Panel to periodically review hair dye epidemiology studies and update this section.

SUMMARY

Basic Blue 99 is a direct, nonoxidative hair colorant used in temporary and semipermanent hair dyes. According to current reported usage data, Basic Blue 99 is used at concentrations from 0.004% to 2% and the most often reported use is in hair tints. Approximately 60% to 63% of the mixture is the Basic Blue 99 dye, whereas the remainder of the mixture is composed of sugar (~25.7%), volatile matter/water crystallization (~1.8%), and inorganic salts (bringing the mixture to 100%).

Hair dyes containing Basic Blue 99, as "coal tar" hair dye products, are exempt from the principal adulteration provision and from the color additive provision 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. Preliminary testing on or by individuals should be done using an open patch test that is evaluated at 48 h after application of the test material. Users, therefore, would be able to determine their individual reactions to hair dye products containing Basic Blue 99.

The data indicated that dermal absorption of Basic Blue 99 is very low (<0.1%) in both rats and humans.

The LD_{50} values of Basic Blue 99 in mice and rats were 2.7 g/kg and between 1.0 g/kg and greater than 2.0 g/kg, respectively. Mice orally administered Basic Blue 99 up to 500 mg/kg/day and rats orally administered up to 360 mg/kg/day for 90 days did not give any indications of cumulative toxicity and no deaths occurred. Discoloration of organs involved in the elimination of Basic Blue 99 from the animals was noted in both test species.

In rabbits, Basic Blue 99 (0.5%) did not cause ocular irritation, but some discoloration was noted. Basic Blue 99

(0.5 g) caused minimal dermal irritation in rabbits. Sensitization occurred in animals exposed to Basic Blue 99 only in a DMSO vehicle. Basic Blue 99 in a water vehicle did not cause dermal sensitization in either species tested (guinea pigs and mice).

Basic Blue 99 (50 mg/kg/day) administered by gavage did not cause developmental toxicity in rats.

Basic Blue 99 is mutagenic with and without metabolic activation in the Ames test, producing both reverse and frameshift mutations. However, Basic Blue 99 did not induce mutations using *Escherichia coli* or show any mutagenic activity in any mammalian cells tested.

Using a modified RIPT test, no volunteers had any reaction to Basic Blue 99 after a 1-h occlusive challenge. Case reports have documented positive patch test results to 1% Basic Blue 99 in three patients.

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 and some 78 studies were considered in 1993 by an International Agency for Research on Cancer (IARC) working group. They concluded that "personal use of hair colourants cannot be evaluated as to its carcinogenicity" and that "occupation as a hairdresser or barber entails exposures that are probably carcinogenic." The IARC report did not distinguish between personal use of oxidative/permanent versus direct hair dyes, or distinguish among the multiple chemical exposures in addition to hair dyes to which a hairdresser or barber might be exposed. In 2003, an updated review of the available epidemiology literature was prepared. This review considered 83 literature citations available since the IARC review and concluded that the available epidemiology studies are insufficient to conclude there is a causal relationship between hair dye use and cancer and other end points described.

Use of direct hair dyes, although not the focus in all investigations, appears to have little evidence of any association with adverse events as reported in epidemiology studies.

DISCUSSION

The CIR Expert Panel was initially concerned with evidence of dermal sensitization. The Panel accounted for the differing results in two LLNA studies as an effect of the type of vehicle used (DMSO or bidistilled water). Moreover, an RIPT study using 54 volunteers in an exaggerated exposure (nine 1-h induction exposures followed by a 1-h rechallenge up to 15 days later) did not cause adverse responses. These data, coupled with the negative results in guinea pigs, led the Panel to conclude that there was not a significant risk of skin sensitization. The Panel expects that individuals will perform the preliminary testing on or by individuals, as described in product labeling, using an open patch test that is evaluated at 48 h after application of the test material, as advised in product labeling. Users, therefore, would be able to determine their individual reactions to hair dye products containing Basic Blue 99.

The Panel stated that use of direct hair dyes, although not the focus in all epidemiology studies, appear to have little evidence of an association with cancer and other adverse events. The low dermal absorption of Basic Blue 99, the weak results in the Ames assays, and the negative mammalian genotoxicity led the Panel to conclude that there was little carcinogenic risk of this direct hair dye.

CONCLUSION

The CIR Expert Panel concluded that Basic Blue 99 is safe as a hair dye ingredient in the practices of use and concentration as described in this safety assessment.

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Concentration of Use by FDA Product Category – Basic Blue 99

Product Category	Maximum Concentration of Use
Hair dyes and colors	0.2%

Information collected in 2022

Table prepared: July 6, 2022