Scientific Committee on Consumer Safety

SCCS

OPINION ON

Basic Blue 99
(COLIPA C059)

Submission III

The SCCS adopted this opinion at its 7\textsuperscript{th} plenary meeting
of 23 September 2014
About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat. They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm
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BACKGROUND

Submission I and II for the hair dye Basic Blue 99 (INCI) (CAS 68123-13-7) (COLIPA No C059) with the chemical name 3-[(4-amino-6-bromo-5,8-dihydro-l-hydroxy-8-imino-5-oxo-2-naphthalenyl)amino]-N,N,N-trimethyl benzenaminium chloride were transmitted in August 1992 and March 2006 respectively by COLIPA.

Basic Blue 99 is a direct hair dye substance in hair dye formulations with a concentration on head of maximum 1.0%. Following Submission II, in September 2011 the Scientific Committee for Consumer Safety (SCCS) concluded that:

"Basic Blue 99 is a mixture of 23-32 substances of varying concentrations as demonstrated by the HPLC analysis of two batches RS2798801 and 74/75. (Figures 1-3) Due to the highly variable composition of Basic Blue 99 in these batches, the safety of Basic Blue 99 cannot be evaluated." (SCCS/1437/11)¹

Based on these conclusions, in July 2014 EFfCI² provided new analytical data (Submission III) on the batches presented by COLIPA in the previous submissions and on other more recent batches.

TERMS OF REFERENCE

(1) In light of the new data provided, does the SCCS consider Basic Blue 99 (C059) safe as a direct hair dye substance in hair dye formulations with a concentration on-head of maximum 1.0%?

(2) Does the SCCS have any further scientific concerns with regard to the use of Basic Blue 99 (C059) in cosmetic products?

¹ http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_068.pdf
² The European Federation for Cosmetic Ingredients
OPINION

1.1 Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name
Basic Blue 99 (INCI name)

3.1.1.2. Chemical names
Benzenaminium, 3-[(4-amino-6-bromo-5,8-dihydro-1-hydroxy-8-imino-5-oxo-2-naphthalenyl)amino]-N,N,N-trimethyl-, chloride (9CI)
3-[(4-amino-6-bromo-5,8-dihydro-1-hydroxy-8-imino-5-oxo-2-naphtyl)amino]-N,N,N-trimethylanilinium chloride (main component),

3.1.1.3. Trade names and abbreviations
COLIPA C 059
Arianor Steel Blue
Arianor Steel Blue 306004
Basic Blue 99
C.I. 56059

3.1.1.4. CAS / EC number
CAS: 68123-13-7
EC: 268-544-3

3.1.1.5. Structural formula

3.1.1.6. Empirical formula
Formula: C_{19}H_{20}BrN_{4}O_{2}^{+} \times \text{Cl}^{-} (main component)

3.1.2. Physical form
Blue black, fine powder

3.1.3. Molecular weight
Molecular weight: 451.8 (as chloride), 416.3 (as cation)
3.1.4. Purity, composition and substance codes

See General comments to physico-chemical characterisation (below)

3.1.5. Impurities / accompanying contaminants

See General comments on Phys-Chem properties (below)

3.1.6. Solubility

/

3.1.7. Partition coefficient (Log Pow)

Log $P_{ow}$: 1.88 (calculated Syracuse Vers. 1.66)

3.1.8. Additional physical and chemical specifications

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>$&gt; 200,^\circ C$ (thermal decomposition)</td>
</tr>
<tr>
<td>Boiling point</td>
<td>/</td>
</tr>
<tr>
<td>Flash point</td>
<td>/</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>/</td>
</tr>
<tr>
<td>Density</td>
<td>/</td>
</tr>
<tr>
<td>Viscosity</td>
<td>/</td>
</tr>
<tr>
<td>pKa</td>
<td>/</td>
</tr>
<tr>
<td>Refractive index</td>
<td>/</td>
</tr>
<tr>
<td>UV_Vis spectrum (200-800 nm)</td>
<td>Absorption maxima were at 268 nm, 576 nm, and 630 nm</td>
</tr>
</tbody>
</table>

3.1.9. Homogeneity and Stability

A freshly prepared sample of Basic Blue 99 batch 0107664 at 0.05 mg/ml in water was compared by LC-DAD with a sample stored 3 days at autosampler conditions (4°C). According to the main peak area, the sample was stable within a period of 3 days at 4°C as a recovery of 99.6 % was found under the study conditions.

General Comments to physico-chemical characterisation

Submission I and II

Purity

Basic Blue 99 is a mixture of 23-32 substances of varying concentrations as demonstrated by the HPLC analysis of two batches RS2798801 and 74/75. (Figures 1-3)

The SCCS is not convinced that all components of Basic Blue 99 (batches RS2798801 and 74/75) are adequately characterised by NMR and IR.

The SCCS considers that the chemical characterisation of individual components of Basic Blue 99 (batches RS2798801 and 74/75) based on LC/MS analysis (UV-Vis spectrum and 1-4 molecular ions) is a poor chemical characterisation.

The HPLC peak areas of other components of Basic Blue 99, characterised by the study authors, are also very different (Tables 1 & 2) in the two batches. In addition, the LC/MS characterisation of the Basic Blue 99 according to the study authors revealed that
the isomeric composition of individual components of the two batches is also different (Tables 1 & 2).

Figure 1: HPLC analysis of Basic Blue 99, Batch RS2798801 (ref.2)
Figure 2: HPLC analysis of Basic Blue 99, Batch 74/75 (Ref. 3)

Sample Amt: 920 mg/l
Run Type: Unknown
Figure 3: Comparison of HPLC chromatograms of Basic Blue 99, batches RS2798801 (upper) and 74/75 (lower)
Table 1: The composition of the two batches (RS2798801 and 74/75) of Basic Blue 99: HPLC peaks of Basic Blue 99 are characterised by names (LC/MS characterisation), and composition of Basic Blue 99 is shown by the area percentage of each component (and their isomers) (Ref. 2, 3).

Batch RS2798801

<table>
<thead>
<tr>
<th>Pk #</th>
<th>Name</th>
<th>Retention Time</th>
<th>Area</th>
<th>Area Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A1</td>
<td>2.807</td>
<td>76469</td>
<td>0.109</td>
</tr>
<tr>
<td>2</td>
<td>A2</td>
<td>3.387</td>
<td>373216</td>
<td>0.530</td>
</tr>
<tr>
<td>3</td>
<td>M1</td>
<td>17.573</td>
<td>62093</td>
<td>0.088</td>
</tr>
<tr>
<td>4</td>
<td>M2</td>
<td>20.060</td>
<td>493322</td>
<td>0.701</td>
</tr>
<tr>
<td>5</td>
<td>D2</td>
<td>20.787</td>
<td>845987</td>
<td>1.201</td>
</tr>
<tr>
<td>6</td>
<td>D3</td>
<td>21.327</td>
<td>25809798</td>
<td>36.655</td>
</tr>
<tr>
<td>7</td>
<td>T2</td>
<td>21.640</td>
<td>223205</td>
<td>0.317</td>
</tr>
<tr>
<td>8</td>
<td>D5</td>
<td>21.867</td>
<td>213419</td>
<td>0.303</td>
</tr>
<tr>
<td>9</td>
<td>M2</td>
<td>22.200</td>
<td>716968</td>
<td>1.018</td>
</tr>
<tr>
<td>10</td>
<td>D5</td>
<td>22.747</td>
<td>362206</td>
<td>0.514</td>
</tr>
<tr>
<td>11</td>
<td>T2</td>
<td>22.960</td>
<td>2940571</td>
<td>4.176</td>
</tr>
<tr>
<td>12</td>
<td>D4</td>
<td>23.347</td>
<td>2846759</td>
<td>4.043</td>
</tr>
<tr>
<td>13</td>
<td>T3</td>
<td>23.547</td>
<td>1259885</td>
<td>1.789</td>
</tr>
<tr>
<td>14</td>
<td>T3</td>
<td>24.247</td>
<td>2926186</td>
<td>4.156</td>
</tr>
<tr>
<td>15</td>
<td>T2</td>
<td>24.787</td>
<td>930366</td>
<td>1.321</td>
</tr>
<tr>
<td>16</td>
<td>D5</td>
<td>25.047</td>
<td>2140861</td>
<td>3.040</td>
</tr>
<tr>
<td>17</td>
<td>D4</td>
<td>25.620</td>
<td>10849541</td>
<td>15.408</td>
</tr>
<tr>
<td>18</td>
<td>D4</td>
<td>26.767</td>
<td>2139409</td>
<td>3.038</td>
</tr>
<tr>
<td>19</td>
<td>D4</td>
<td>27.307</td>
<td>4702229</td>
<td>6.678</td>
</tr>
<tr>
<td>20</td>
<td>T5</td>
<td>27.647</td>
<td>812561</td>
<td>1.154</td>
</tr>
<tr>
<td>21</td>
<td>T5</td>
<td>28.140</td>
<td>1275192</td>
<td>1.811</td>
</tr>
<tr>
<td>22</td>
<td>T5</td>
<td>28.373</td>
<td>53318</td>
<td>0.076</td>
</tr>
<tr>
<td>23</td>
<td>T5</td>
<td>28.760</td>
<td>444057</td>
<td>0.631</td>
</tr>
<tr>
<td>24</td>
<td>T5</td>
<td>29.313</td>
<td>4458174</td>
<td>6.331</td>
</tr>
<tr>
<td>25</td>
<td>N1</td>
<td>29.740</td>
<td>1580425</td>
<td>2.244</td>
</tr>
<tr>
<td>26</td>
<td>T6</td>
<td>30.693</td>
<td>56413</td>
<td>0.080</td>
</tr>
<tr>
<td>27</td>
<td>T7</td>
<td>31.813</td>
<td>684980</td>
<td>0.973</td>
</tr>
<tr>
<td>28</td>
<td>T5</td>
<td>32.700</td>
<td>429202</td>
<td>0.610</td>
</tr>
<tr>
<td>29</td>
<td>T5</td>
<td>33.200</td>
<td>399826</td>
<td>0.568</td>
</tr>
<tr>
<td>30</td>
<td>T5</td>
<td>33.973</td>
<td>139394</td>
<td>0.198</td>
</tr>
<tr>
<td>31</td>
<td>T5</td>
<td>37.713</td>
<td>84265</td>
<td>0.120</td>
</tr>
<tr>
<td>32</td>
<td>T5</td>
<td>38.680</td>
<td>83027</td>
<td>0.118</td>
</tr>
</tbody>
</table>

| Totals | 70413324 | 100.000 |
Table 2: Distribution of major components (and their isomers) of Basic Blue 99 in the batches RS2798801 and 74/75, deduced from Table 1

<table>
<thead>
<tr>
<th>Component/Name</th>
<th>Peak No. of all isomers</th>
<th>Area percent of all isomers</th>
<th>Component/Name</th>
<th>Peak No. of all isomers</th>
<th>Area percent of all isomers</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3</td>
<td>6</td>
<td>36.7%</td>
<td>D3</td>
<td>11</td>
<td>49.3%</td>
</tr>
<tr>
<td>D4</td>
<td>12, 17, 19</td>
<td>4.0+15.4+6.7 = 26.1%</td>
<td>D4</td>
<td>19, 21</td>
<td>2.6+10.9 = 13.5%</td>
</tr>
<tr>
<td>D5</td>
<td>8, 10, 16</td>
<td>0.3+0.5+3.0 = 3.8%</td>
<td>D5</td>
<td>20</td>
<td>1.3%</td>
</tr>
<tr>
<td>T2</td>
<td>7, 11, 15</td>
<td>0.3+4.2+1.3 = 5.8%</td>
<td>T2</td>
<td>14</td>
<td>1.6%</td>
</tr>
<tr>
<td>T3</td>
<td>13, 14</td>
<td>1.8+4.2 = 6.0%</td>
<td>T3</td>
<td>16, 18</td>
<td>1.8+10.9 = 12.7%</td>
</tr>
<tr>
<td>T5</td>
<td>21, 24, 28</td>
<td>1.2+6.3+0.6 = 8.1%</td>
<td>T5</td>
<td>22</td>
<td>1.4%</td>
</tr>
</tbody>
</table>

Information on purity (and impurity) of Basic Blue 99, according to Submission III, 2014

Purity
Purity (% HPLC): >48 area-%
Sum of 3 isomers with 3-[(4-amino-6-bromo-5,8-dihydro-1-hydroxy-8-imino-5-oxo-2-naphtyl)amino]-N,N,Ntrimethylanilinium chloride as main isomer, according to 1H-NMR.
Water content (Karl Fisher): <7 weight-%
Chloride (as Cl- %): <33 weight-%
Sulfate (as SO42-) %: <2 weight-%
Acetate (as CH3COO-) %: <2 weight-%
Water insoluble matter %: <5 weight-%
Zinc: <7 weight-%

Table 3: Analytical description of Batches used in Toxicity studies or actual market materials

<table>
<thead>
<tr>
<th>ID</th>
<th>Structure</th>
<th>MW</th>
<th>Peak no.</th>
<th>Range (area%)</th>
<th>RS27988101 (area-%)</th>
<th>125 (area-%)</th>
<th>140 (area-%)</th>
<th>106106 (area-%)</th>
<th>107664 (area-%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main</td>
<td><img src="image" alt="Structure" /></td>
<td>415 / 417</td>
<td>10, 13, 14</td>
<td>&gt;48</td>
<td>62.8</td>
<td>50.2</td>
<td>48.2</td>
<td>57.3</td>
<td>64.1</td>
</tr>
</tbody>
</table>

Table 4: Subsidiary colours:
Members of an isomer set whose total percentage area (area-%) is greater than 1.0% at 500-700 nm and are considered to contribute to the desired blue coloration of hair have been classified as Subsidiary Colours.
Identity was verified for each batch by UV and IR spectroscopy. Before marketing of Basic Blue 99, sodium chloride and/or saccharose are usually added to the neat dye in order to adjust the colour strength to a certain predefined value.

**Impurity**

Table 4: Organic impurities
Members of an isomer set lacking one or both of the criteria mentioned in the purity section above
Inorganic impurities
Pb <20 ppm; Sb and Ni <10 ppm; As and Cd <5 ppm; Hg <1 ppm

The purity of Basic Blue 99 based on major components (≥5% HPLC peak area) can be reported as described in Table 5 below.

Table 5: Purity of Basic Blue 99 (main component + subsidiary colours)

<table>
<thead>
<tr>
<th>Basic Blue 99 component</th>
<th>No. of isomers</th>
<th>%HPLC peak area (Range)</th>
<th>Isomer composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Main component</td>
<td>3</td>
<td>48.0 - 67.8</td>
<td>Not known</td>
</tr>
<tr>
<td>F</td>
<td>8</td>
<td>7.0 - 26.5</td>
<td>Not known</td>
</tr>
<tr>
<td>E</td>
<td>3</td>
<td>4.4 – 15.0</td>
<td>Not known</td>
</tr>
<tr>
<td>A</td>
<td>2</td>
<td>0.0 – 9.5</td>
<td>Not known</td>
</tr>
<tr>
<td>J</td>
<td>1</td>
<td>2.0 - 6.0</td>
<td>Not known</td>
</tr>
<tr>
<td>L</td>
<td>2</td>
<td>1.3 – 5.0</td>
<td>Not known</td>
</tr>
</tbody>
</table>

*It is clear from Table 2 that isomeric composition of various components may also vary from batch to batch

It is obvious from Table 5 that composition of Basic Blue 99 varies significantly from batch to batch.

The physico-chemical properties as well as biological activity of a mixture will depend upon the composition of the mixture. As the six batches of Basic Blue 99 were demonstrated to be a mixture of up to 40 substances of varying composition (and varying isomeric composition), the safety of such a mixture cannot be assessed.
3.2. Function and uses

Basic Blue 99 is used as a direct hair dye substance in hair dye formulations with a maximum on-head concentration of 1.0%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

Not applicable

3.3.2. Irritation and corrosivity

Not applicable

3.3.3. Skin sensitisation

Not applicable

3.3.4. Dermal / percutaneous absorption

Not applicable

3.3.5. Repeated dose toxicity

Not applicable

3.3.6. Mutagenicity / Genotoxicity

Not applicable

3.3.7. Carcinogenicity

Not applicable

3.3.8. Reproductive toxicity

Not applicable

3.3.9. Toxicokinetics

Not applicable
Opinion on Basic Blue 99

3.3.10. Photo-induced toxicity
Not applicable

3.3.11. Human data
Not applicable

3.3.12. Special investigations
Not applicable

3.3.13. Safety evaluation (including calculation of the MoS)
Not applicable

3.3.14. Discussion
Not applicable

4. CONCLUSION

Basic Blue 99 is a mixture of up to 40 substances of varying concentrations as demonstrated by the HPLC analysis of six batches (See Figures 1-3 and Tables 2, 3 and 5).

Due to the highly variable composition of Basic Blue 99 in six batches, the safety of Basic Blue 99 cannot be evaluated.

5. MINORITY OPINION

Not applicable
6. REFERENCES

15. Ward, R.J. (2002). In vitro penetration of BASIC BLUE 99 through pig skin from a vehicle and a standard formulation. Central Toxicology Laboratory, Cheshire, UK, internal Study No. JV1718. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0300002

Submission III July 2014