SCCS OPINION ON Basic Blue 99 (C059)
Ulrike Bernauer, Laurent Bodin, Leonardo Celleno, Qasim Mohammad Chaudhry, Pieter-Jan Coenraads, Maria Dusinska, Janine Ezendam, Eric Gaffet, Corrado Lodovico Galli, Berit Brunstad Granum, et al.

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Scientific Committee on Consumer Safety

SCCS

OPINION ON
Basic Blue 99 (C059)

The SCCS adopted this Opinion on 7 March 2017
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SCCS

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CAS: 68123-13-7, EC: 268-544-3

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1. BACKGROUND

The substance Basic Blue 99 (INCI) (CAS 68123-13-7) (COLIPA No C059) with the chemical name 3-[(4-amino-6-bromo-5,8-dihydro-1-hydroxy-8-imino-5-oxo-2-naphthalenyl)amino]-N,N,N-trimethyl benzenaminium chloride is a direct hair dye substance in hair dye formulations with a concentration on-head of maximum 1.0%.

Submission I and II for the hair dye Basic Blue 99 were transmitted in August 1992 and March 2006 respectively by COLIPA. Following Submission II, in September 2011 the Scientific Committee for Consumer Safety (SCCS) expressed concerns regarding the highly variable composition of Basic Blue of the analysed batches that made it impossible to conclude on the safety of the substance (SCCS/1437/11).

In reply to these scientific concerns, in July 2014 EFfCI provided new analytical data (Submission III) on the batches presented by COLIPA in the previous submissions and on other more recent batches. In September 2014, the SCCS concluded that:

"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." (SCCS/1537/14).

In April 2016, EFfCI submitted another dossier (Submission IV) containing new information on composition in an update of the analytical description of market quality and other data.

2. TERMS OF REFERENCE

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

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

3.1 **Chemical and Physical Specifications**

3.1.1 **Chemical identity**

3.1.1.1 Primary name and/or INCI name

Basic Blue 99

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

C059

Arianor Steel Blue

Jarocol Steel Blue

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-[(4-amino-6-bromo-1-hydroxy-8-imino-5-oxo-5,8-dihydrophthalen-2-yl)aminol-N,N,N-trimethylbenzenaminium chloride

3.1.1.6 Empirical formula

Formula: C19H20BrN4O2+ x 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 to physico-chemical characterisation (below)

3.1.6 Solubility

Water 10-100 g/L room temperature
Ethanol 1-10 g/L room temperature
DMSO 1-10 g/L room temperature

3.1.7 Partition coefficient (Log \( P_{ow} \))

Log Pow: 1.88 (calculated with Syracuse)

3.1.8 Additional physical and chemical specifications

Melting point: > 200 °C (thermal decomposition)
Boiling point: /
Flash point: /
Vapour pressure: /
Density: /
Viscosity: /
pKa: /
Refractive index: /
UV_Vis spectrum (200-800 nm): /

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 HPLC-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 the physicochemical part

The provided data and the SCCS comments according to Submissions I, II and III are summarised in Annexes I and II
Information on purity (and impurity) of Basic Blue 99, according to Submission IV, 2016

Purity

According to the applicant, the product is a mixture of the following defined structures:

1. \( (1) = 3-[(\text{Bromo-8-amino-5-hydroxy-4-imino-1-oxo-1,4-dihyronaphthalenyl})\text{amino}]\)-N,N,N trimethylbenzeneaminium chloride
2. \( (2) = 3-[(\text{Bromo-8-amino-5-hydroxy-1,4-dioxo-1,4-dihyronaphthalenyl})\text{amino}]\)-N,N,N trimethylbenzeneaminium chloride
3. \( (3) = 3-[(\text{Dibromo-8-amino-5-hydroxy-4-imino-1-oxo-1,4-dihyronaphthalenyl})\text{amino}]\)-N,N,N trimethylbenzeneaminium chloride
4. \( (4) = 3-[(\text{Bromo-5,8-dihydroxy-1,4-dioxo-1,4-dihydronaphthalenyl})\text{amino}]\)-N,N,N trimethylbenzeneaminium chloride

The purity of Basic Blue 99, based on major components (≥ 5% HPLC peak area) linked to batch 74/75 used in toxicity studies and to updated representative market materials, is described in Table 1 (HPLC results).

**Table 1:** Purity of Basic Blue 99 based on major components (≥ 5% HPLC peak area)

<table>
<thead>
<tr>
<th>ID</th>
<th>MW (as cation)</th>
<th>74/75</th>
<th>106100</th>
<th>20150121</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>337</td>
<td>1.7</td>
<td>1.7</td>
<td>2.8</td>
<td>1.0-4.0</td>
</tr>
<tr>
<td>B</td>
<td>418</td>
<td>1.8</td>
<td>3.8</td>
<td>0.8</td>
<td>0.0-4.0</td>
</tr>
<tr>
<td>C</td>
<td>338</td>
<td>0.4</td>
<td>0.2</td>
<td>1.4</td>
<td>0.0-2.0</td>
</tr>
<tr>
<td>E</td>
<td>493/495/497</td>
<td>11.8</td>
<td>7.4</td>
<td>6.1</td>
<td>6.0-12.0</td>
</tr>
<tr>
<td>F</td>
<td>416/418</td>
<td>8.4</td>
<td>7.0</td>
<td>12.5</td>
<td>7.0-14.0</td>
</tr>
<tr>
<td>G</td>
<td>494/496/498</td>
<td>2.1</td>
<td>2.7</td>
<td>1.8</td>
<td>1.0-3.0</td>
</tr>
<tr>
<td>J</td>
<td>417/419</td>
<td>4.1</td>
<td>5.4</td>
<td>3.5</td>
<td>3.0-6.0</td>
</tr>
<tr>
<td>K</td>
<td>430/432</td>
<td>0.9</td>
<td>0.0</td>
<td>0.8</td>
<td>0.0-2.0</td>
</tr>
<tr>
<td>L</td>
<td>423/429</td>
<td>2.6</td>
<td>4.4</td>
<td>3.0</td>
<td>2.0-5.0</td>
</tr>
<tr>
<td>M</td>
<td>495/497</td>
<td>0.9</td>
<td>0.0</td>
<td>0.6</td>
<td>0.0-1.0</td>
</tr>
</tbody>
</table>
Based on these results, the applicant narrowed Basic Blue 99 composition definition (see Table 2).

### Table 2: Composition definition of Basic Blue 99

<table>
<thead>
<tr>
<th>Basic Blue 99 components</th>
<th>MW (as cation)</th>
<th>% HPLC peak area (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Chemical structure" /></td>
<td>416.3</td>
<td>58.0 – 70.0</td>
</tr>
<tr>
<td><img src="image" alt="Chemical structure" /></td>
<td>417.3</td>
<td>7.0 – 14.0</td>
</tr>
<tr>
<td><img src="image" alt="Chemical structure" /></td>
<td>495.2</td>
<td>6.0 – 12.0</td>
</tr>
<tr>
<td><img src="image" alt="Chemical structure" /></td>
<td>418.3</td>
<td>3.0 – 6.0</td>
</tr>
</tbody>
</table>

**Impurity**

Inorganic impurities:
Pb <20 ppm; Sb and Ni <10 ppm; As and Cd <5 ppm; Hg <1 ppm

Organic impurities: Subsidiary colours (HPLC peak area below 5% and above 1%) and impurities (HPLC peak area below 1%) are summarised in Table 3:
**Table 3**: Organic impurities based on HPLC data (HPLC peak area below 5% and above 1%) and impurities (HPLC peak area below 1%)

<table>
<thead>
<tr>
<th>Basic Blue 99 subsidiary colors</th>
<th>MW (as cation)</th>
<th>% HPLC peak area (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(K)</td>
<td>431.3</td>
<td>0.0 - 2.0</td>
</tr>
<tr>
<td>(L)</td>
<td>425.9</td>
<td>2.0 - 5.0</td>
</tr>
<tr>
<td>(M)</td>
<td>497.4</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>(N)</td>
<td>496.4</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>(G)</td>
<td>338.4</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>(B)</td>
<td>418.5</td>
<td>0.0 - 4.0</td>
</tr>
<tr>
<td>(O)</td>
<td>417.5</td>
<td>0.0 - 2.0</td>
</tr>
<tr>
<td>(C)</td>
<td>338.4</td>
<td>0.0 - 2.0</td>
</tr>
</tbody>
</table>

**Isomer composition**

Compounds identified in Hair Dye C059 are summarised in table 4.
Table 4: Compounds identified in Hair Dye C059

<table>
<thead>
<tr>
<th>ID</th>
<th>Chemical name</th>
<th>General structure</th>
<th>Role</th>
<th>N° of isomers</th>
<th>Expected main isomer</th>
<th>MW</th>
<th>λ max (nm)</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3-[(Bromo-5-amino-5-hydroxy-4-amino-1,4-dihydronaphthaleny]amino]-N,N,N-trimethylbenzenamine chloride</td>
<td>Main component</td>
<td>3</td>
<td>415/417</td>
<td></td>
<td>625, 560</td>
<td>C18H12BrN3O2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3-[(6-Amino-5-hydroxy-4-amino-1,4-dihydronaphthaleny]amino]-N,N,N-trimethylbenzenamine chloride</td>
<td>Subsidiary colour</td>
<td>2</td>
<td>357</td>
<td></td>
<td>603, 561</td>
<td>C18H21N4O2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3-[(6-Iodo-8-methylene-1,4-dihydronaphthaleny]amino]-N,N,N-trimethylbenzenamine chloride</td>
<td>Subsidiary colour</td>
<td>1</td>
<td>418</td>
<td></td>
<td>593 (sh), 507</td>
<td>C19H21N3O3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3-[(6-Amino-5-hydroxy-1,4-dihydronaphthaleny]amino]-N,N,N-trimethylbenzenamine chloride</td>
<td>Subsidiary colour</td>
<td>1</td>
<td>338</td>
<td></td>
<td>512</td>
<td>C18H21N3O3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3-[(8-Amino-5-hydroxy-1,4-dihydronaphthaleny]amino]-N,N,N-trimethylbenzenamine chloride</td>
<td>Main component</td>
<td>3</td>
<td>460/497</td>
<td></td>
<td>623, 574</td>
<td>C19H18N3O2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3-[(Bromo-8-amino-5-hydroxy-1,4-dihydronaphthaleny]amino]-N,N,N-trimethylbenzenamine chloride</td>
<td>Main component</td>
<td>6</td>
<td>416/418</td>
<td></td>
<td>595, 557</td>
<td>C19H18BrN3O2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3-[(Bromo-8-amino-5-hydroxy-1,4-dihydronaphthaleny]amino]-N,N,N-trimethylbenzenamine chloride</td>
<td>Subsidiary colour</td>
<td>3</td>
<td>494/495</td>
<td></td>
<td>628, 585</td>
<td>C19H18BrN3O2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3-[(Bromo-5,8-dihydroxy-1,4-dihydronaphthaleny]amino]-N,N,N-trimethylbenzenamine chloride</td>
<td>Main component</td>
<td>1</td>
<td>417/419</td>
<td></td>
<td>623, 574</td>
<td>C19H18BrN3O2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>3-[(Bromo-8-amino-5-hydroxy-1,4-dihydronaphthaleny]amino]-N,N,N,N-trimethylbenzenamine chloride</td>
<td>Impurity</td>
<td>1</td>
<td>430/432</td>
<td></td>
<td>—</td>
<td>C19H20N3O2</td>
<td></td>
</tr>
</tbody>
</table>
Based on the provided chemical structures for the compounds 1 to 4, compounds 1, 2 and 3 have three isomers, while compound 4 has 6 isomers.

It is obvious from Table 2 that the composition of Basic Blue 99 varies from 58 to 70% from batch to batch. In addition, when compared with the previous batches, purity data for the batches RS 2798801 (50.2%), 125 (48.2%) and 140 (57.3%) and 107664 (67.8%) have been excluded from this Table by the Applicant.

The physicochemical properties and the biological activity of a chemical mixture will depend upon the composition of the mixture. The data on chemical analysis of six batches of Basic Blue 99 has demonstrated a highly variable composition of the material and has shown that it can be a mixture of up to 40 chemical analogues as well as several isomeric forms of some of them. Safety assessment will need toxicological data that are representative of the batch-to-batch variability.

### 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

#### 3.3.1.1 Acute oral toxicity
3.3.1.2 Acute dermal toxicity

3.3.1.3 Acute inhalation toxicity

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

3.3.2.2 Mucous membrane irritation / Eye irritation

3.3.3 Skin sensitisation

3.3.4 Dermal / percutaneous absorption

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (14 days) oral toxicity

3.3.5.2 Sub-chronic (90 days) toxicity (oral)

3.3.5.3 Chronic (> 12 months) toxicity

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity in vitro

3.3.6.2 Mutagenicity / Genotoxicity in vivo

3.3.7 Carcinogenicity

3.3.8 Reproductive toxicity

3.3.8.1 Two-generation reproduction toxicity

3.3.8.2 Other data on fertility and reproduction toxicity
3.3.8.3 Developmental Toxicity

3.3.9 Toxicokinetics

3.3.10 Photo-induced toxicity

3.3.11 Human data

3.3.12 Special investigations

3.3.13 Safety evaluation (including calculation of the MoS)

3.3.14 Discussion

The data provided as part of the submission has indicated that the material is not composed of a single substance, but of different substances and isomers. Analysis of different batches has shown that there is a large variation in the composition of the material intended for commercial use. Also, the toxicological data provided in the previous submission do not relate to the material specifications provided for the current assessment.

4. CONCLUSION

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

The SCCS cannot conclude on the safety of Basic Blue 99 (C059) because it is composed of several substances and isomeric forms, with a large variability between the composition of different batches. Also, the toxicological data provided in the previous submission do not relate to the material specifications provided for the current assessment. 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.

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

/ 

5. MINORITY OPINION

/
6. REFERENCES


7. ANNEX

ANNEX I : Basic Blue 99 Submission I and II: Summary on the physico-chemical characterisation (provided data and SCCS comments)

Summary of the provided data on the composition of the Basic Blue 99 are listed in Tables 1 & 2 below.

Table 1: The composition of the two batches (RS2798801 and 74/75) of Basic Blue 99*

<table>
<thead>
<tr>
<th>Batch No</th>
<th>HPLC-PDA data</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Batch RS2798801</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td><strong>A1</strong></td>
</tr>
<tr>
<td>2</td>
<td><strong>A2</strong></td>
</tr>
<tr>
<td>3</td>
<td><strong>A3</strong></td>
</tr>
<tr>
<td>4</td>
<td><strong>A4</strong></td>
</tr>
<tr>
<td>5</td>
<td><strong>A5</strong></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
</tr>
</tbody>
</table>

| Batch 74/75 | |
| 1 | **B1** | 2.706 | 109410 | 0.009 |
| 2 | **B2** | 3.700 | 277482 | 0.625 |
| 3 | **B3** | 3.700 | 277482 | 0.375 |
| 4 | **B4** | 5.700 | 277482 | 0.000 |
| 5 | **B5** | 5.700 | 277482 | 0.000 |
| **Total** | | 109410 | 1.000 |

*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).

Table 2: Distribution of major components (and their isomers) of Basic Blue 99 in the batches RS2798801 and 74/75, deduced from Table 1.
SCCS general comments on 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 (Table 1). 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 area of the major component of Basic Blue 99 in the two batches (batches RS2798801 and 74/75) 36% and 49% (Tables 1 &2), is significantly different from each other. 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).
ANNEX II: Basic Blue 99 Submission III (2014): Summary on the physico-chemical characterisation (provided data and SCCS comments)

Provided data on Purity
Basic Blue 99 is a sum of 3 isomers with 3-[(4-amino-6-bromo-5,8-dihydro-1-hydroxy-8-imino-5-oxo-2-naphtyl)amino]-N,N,N trimethylanilinium chloride as main isomer, according to 1H-NMR. Purity (% HPLC): >48 area-% (Table 1)

Table 1: 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>74/75</th>
<th>R52/78/83/131</th>
<th>R52/78/82/01</th>
<th>R52/105/01</th>
<th>140</th>
<th>R101/06</th>
<th>R107/06</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td></td>
<td>415</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>
<td>67.8</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>468</td>
<td>18, 20, 22</td>
<td>≤15</td>
<td>11.8</td>
<td>14.6</td>
<td>4.4</td>
<td>5.5</td>
<td>7.4</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>337</td>
<td>1, 5</td>
<td>&lt;9.5</td>
<td>1.7</td>
<td>9.3</td>
<td>1.6</td>
<td>0.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J</td>
<td></td>
<td>417</td>
<td>30</td>
<td>≤6.0</td>
<td>4.1</td>
<td>4.8</td>
<td>2.0</td>
<td>3.4</td>
<td>5.4</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td></td>
<td>425</td>
<td>38, 40</td>
<td>≤5.0</td>
<td>2.60</td>
<td>1.5</td>
<td>1.7</td>
<td>1.5</td>
<td>4.4</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>418</td>
<td>4, 11</td>
<td>≤4.0</td>
<td>1.80</td>
<td>2.0</td>
<td>2.8</td>
<td>2.2</td>
<td>3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td></td>
<td>454</td>
<td>31, 32, 34</td>
<td>≤3.0</td>
<td>2.10</td>
<td>2.8</td>
<td>1.2</td>
<td>0.8</td>
<td>2.7</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>496</td>
<td>3</td>
<td>≤3.0</td>
<td>1.0</td>
<td>--</td>
<td>2.0</td>
<td>2.8</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td>499</td>
<td>21</td>
<td>≤1.7</td>
<td>--</td>
<td>1.7</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td></td>
<td>495</td>
<td>7</td>
<td>≤1.6</td>
<td>0.9</td>
<td>--</td>
<td>1.3</td>
<td>--</td>
<td>--</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Provided data on 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 (Table 2)

Table 2: 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: organic impurities are presented in Table 3. Members of an isomer set lacking one or both of the criteria mentioned in the purity section above

Table 3: Organic impurities of Basic Blue 99

<table>
<thead>
<tr>
<th>ID</th>
<th>Structure</th>
<th>MW</th>
<th>Peak no.</th>
<th>Range (area %)</th>
<th>74/75 (area-%)</th>
<th>R227986/01 (area-%)</th>
<th>125 (area-%)</th>
<th>410 (area-%)</th>
<th>106106 (area-%)</th>
<th>107664 (area-%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td><img src="Structure1.png" alt="Structure" /></td>
<td>451</td>
<td>6</td>
<td>≤1.3</td>
<td>---</td>
<td>---</td>
<td>1.3</td>
<td>0.2</td>
<td>0.6</td>
<td>---</td>
</tr>
<tr>
<td>C</td>
<td><img src="Structure2.png" alt="Structure" /></td>
<td>338</td>
<td>8</td>
<td>≤1.2</td>
<td>0.4</td>
<td>0.6</td>
<td>---</td>
<td>1.2</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>K</td>
<td><img src="Structure3.png" alt="Structure" /></td>
<td>435/432</td>
<td>29</td>
<td>≤0.9</td>
<td>0.9</td>
<td>---</td>
<td>---</td>
<td>0.4</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Q</td>
<td><img src="Structure4.png" alt="Structure" /></td>
<td>352</td>
<td>17</td>
<td>≤0.5</td>
<td>0.2</td>
<td>0.5</td>
<td>0.1</td>
<td>---</td>
<td>0.2</td>
<td>---</td>
</tr>
</tbody>
</table>

--- Not known
--- Not known

Inorganic impurities: Pb <20 ppm; Sb and Ni <10 ppm; As and Cd <5 ppm; Hg <1 ppm

Purity Based on major components: The purity of Basic Blue 99 based on major components (≥5% HPLC peak area) can be reported as described in Table 4.

Table 4: 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

SCCS comments: 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.