Scientific Committee on Consumer Safety

SCCS

OPINION ON
Basic Blue 99 (C059)

The SCCS adopted this Opinion
on 6 June 2017

About the Scientific Committees
Two 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 that may pose an actual or potential threat.
These Committees are the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and they are made up of scientists appointed in their personal capacity.

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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The Opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The Opinions are published by the European Commission in their original language only.

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

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SCCS members listed below are acknowledged for their valuable contribution to this Opinion.

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All Declarations of Working Group members are available on the following webpage:
http://ec.europa.eu/health/scientific_committees/experts/declarations/sccs_en.htm

Keywords: SCCS, scientific opinion, hair dye, Basic Blue 99 (C059), Regulation 1223/2009, CAS: 68123-13-7, EC: 268-544-3

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Basic Blue 99 (C059), 7 March 2017, SCCS/1585/17

This opinion has been subject to a commenting period of 9 weeks (from 12 March 2017 to 14 May 2017) after its initial publication.
There were no comments received and the final version of the opinion remained unchanged compared to the preliminary one.

TABLE OF CONTENTS

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

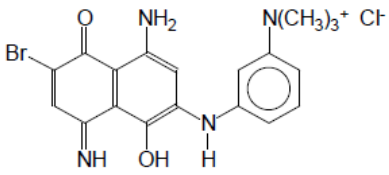
C059

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-dihydronaphthalen-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

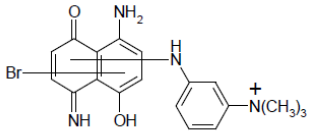
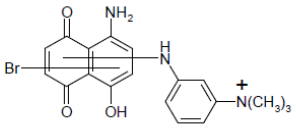
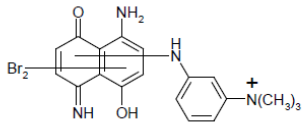
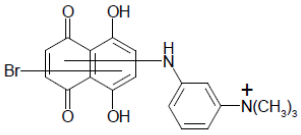
A freshly prepared sample of Basic Blue 99 batch 0107664 at 0.05 mg/ml in water was compared by HPLC-DAD with a sample stored 3 days at autosampler conditions (4°C). According to the main peak area, the sample was stable within a period of 3 days at 4°C, as a recovery of 99.6 % was found under the study 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) (2) (3) (4)

(1) = 3-[(Bromo-8-amino-5-hydroxy-4-imino-1-oxo-1,4-dihydronaphthalenyl)amino]-N,N,N trimethylbenzeneaminium chloride
(2) = 3-[(Bromo-8-amino-5-hydroxy-1,4-dioxo-1,4-dihydronaphthalenyl)amino]-N,N,Ntrimethylbenzeneaminium chloride
(3) = 3-[(Dibromo-8-amino-5-hydroxy-4-imino-1-oxo-1,4-dihydronaphthalenyl)amino]-N,N,Ntrimethylbenzeneaminium chloride
(4) = 3-[(Bromo-5,8-dihydroxy-1,4-dioxo-1,4-dihydronaphthalenyl)amino]-N,N,N trimethylbenzeneaminium chloride

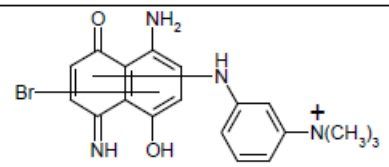
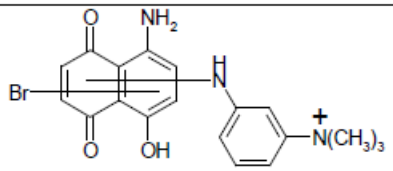
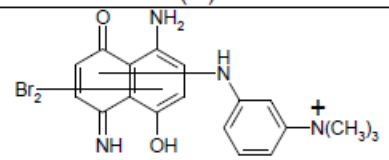
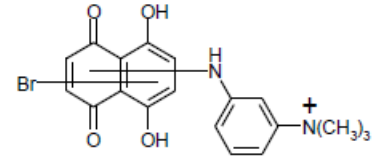
The purity of Basic Blue 99, based on major components (≥ 5% HPLC peak area) linked to batch 74/75 used in toxicity studies and to updated representative market materials, is described in Table 1 (HPLC results).

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

ID	MW (as cation)	74/75	106106	201501121	**Range**
Main	415/417	62.8	64.1	61.3	58.0-70.0
A	337	1.7	1.7	2.8	1.0-4.0
B	418	1.8	3.8	0.8	0.0-4.0
C	338	0.4	0.2	1.4	0.0-2.0
E	493/ 495/ 497	11.8	7.4	6.1	6.0-12.0
F	416/418	8.4	7.0	12.5	7.0-14.0
G	494/ 496/ 498	2.1	2.7	1.8	1.0-3.0
J	417/419	4.1	5.4	3.5	3.0-6.0
K	430/432	0.9	0.0	0.8	0.0-2.0
L	423/429	2.6	4.4	3.0	2.0-5.0
M	495/497	0.9	0.0	0.6	0.0-1.0
N	496/498	0.7	0.0	0.7	0.0-1.0
O	417	1.0	0.0	1.3	0.0-2.0
P	451	0.0	0.6	0.0	0.0-1.0
Q	352	0.2	0.2	0.3	0.0-1.0

Based on these results, the applicant narrowed Basic Blue 99 composition definition (see Table 2).

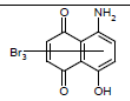
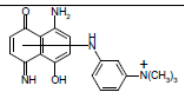
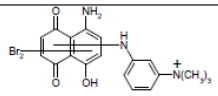
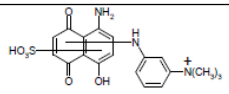
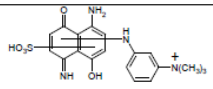
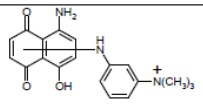
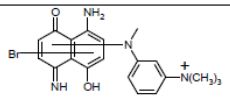
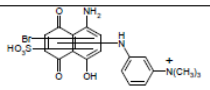
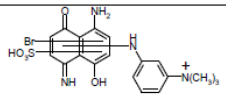
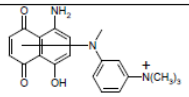
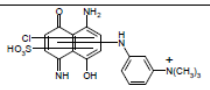
Table 2: Composition definition of Basic Blue 99

Basic Blue 99 components	MW (as cation)	% HPLC peak area (range)
	416.3	58.0 – 70.0
 (F)	417.3	7.0 – 14.0
 (E)	495.2	6.0 – 12.0
 (J)	418.3	3.0 – 6.0

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:

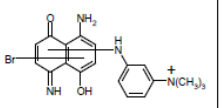
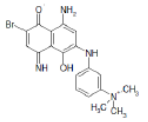
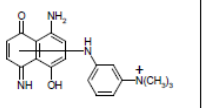
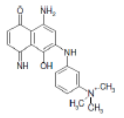
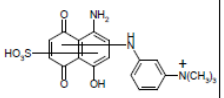
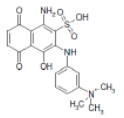
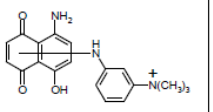
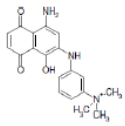
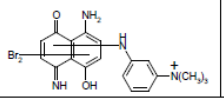
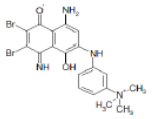
Table 3: Organic impurities based on HPLC data (HPLC peak area below 5% and above 1%) and impurities (HPLC peak area below 1%)

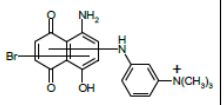
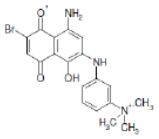
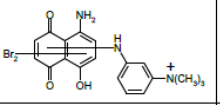
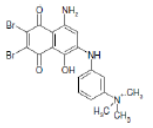
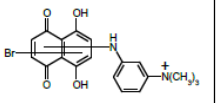
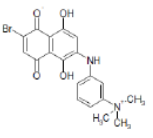
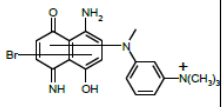
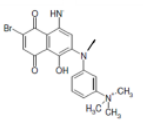
Basic Blue 99 subsidiary colors	MW (as cation)	% HPLC peak area (range)
 (L)	425.9	2.0 - 5.0
 (A)	337.4	1.0 – 4.0
 (G)	496.2	1.0 - 3.0
 (B)	418.5	0.0 - 4.0
 (O)	417.5	0.0 - 2.0
 (C)	338.4	0.0 - 2.0
 (K)	431.3	0.0 - 2.0
 (N)	497.4	< 1.0
 (M)	496.4	< 1.0
 (Q)	338.4	< 1.0
 (P)	451.9	< 1.0

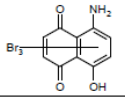
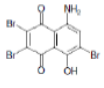
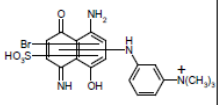
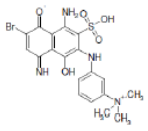
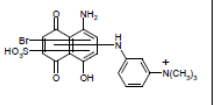
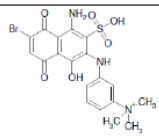
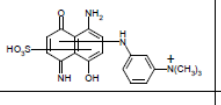
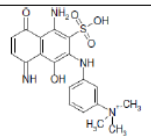
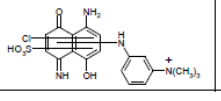
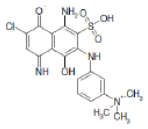
Isomer composition

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

Table 4: Compounds identified in Hair Dye C059

ID	Chemical name	General structure	Role	N° of isomers	Expected main isomer	MW	λ max(nm)	Molecular formula
Main	3-[(Bromo-8-amino-5-hydroxy-4-imino-1-oxo-1,4-dihydronaphthalenyl)amino] -*N,N,N*- trimethylbenzeneaminium chloride		Main component	3		415/417	625, 580	C19H20BrN4O2
A	3-[(8-Amino-5-hydroxy-4-imino-1-oxo-1,4-dihydronaphthalenyl)amino] -*N,N,N*- trimethylbenzeneaminium chloride		Subsidiary colour	2		337	603, 561	C19H21N4O2
B	3-[(Sulpho-8-amino-5-hydroxy-1,4-dioxo-1,4-dihydronaphthalenyl)amino] -*N,N,N*- trimethylbenzeneaminium chloride		Subsidiary colour	1		418	593 (sh), 557	C19H20N3O6S
C	3-[(8-Amino-5-hydroxy-1,4-dioxo-1,4-dihydronaphthalenyl)amino] -*N,N,N*- trimethylbenzeneaminium chloride		Subsidiary colour	1		338	512	C19H20N3O3
E	3-[(Dibromo-8-amino-5-hydroxy-4-imino-1-oxo-1,4-dihydronaphthalenyl)amino] -*N,N,N*- trimethylbenzeneaminium chloride		Main component	3		493/ 495/ 497	623, 574	C19H19Br2N4O2

ID	Chemical name	General structure	Role	N° of isomers	Expected main isomer	MW	λ max(nm)	Molecular formula
F	3-[(Bromo-8-amino-5-hydroxy-1,4-dioxo-1,4-dihydronaphthalenyl)amino] -*N,N,N*- trimethylbenzeneaminium chloride		Main component	6		416/418	595, 557	C19H19BrN3O3
G	3-[(Dibromo-8-amino-5-hydroxy-1,4-dioxo-1,4-dihydronaphthalenyl)amino] -N,N,N- trimethylbenzeneaminium chloride		Subsidiary colour	3		494/496 /498	628, 585	C19H18Br2N3O3
J	3-[(Bromo-5,8-dihydroxy-1,4-dioxo-1,4-dihydronaphthalenyl)amino] -N,N,N- trimethylbenzeneaminium chloride		Main component	1		417/419	623, 574	C19H18BrN2O4
K	3-[(Bromo-8-amino-5-hydroxy-4-imino-1-oxo-1,4-dihydro-naphthalenyl)amino]-3-methyl-N,N,N-trimethylbenzeneaminium chloride		Impurity	1		430/432	---	C20H21BrN3O3

ID	Chemical name	General structure	Role	N° of isomers	Expected main isomer	MW	λ max(nm)	Molecular formula
L	Tribromo-8-amino-5-hydroxy-1,4-naphthoquinone		Subsidiary colour	2		423/429	628, 585	C10H4Br3NO3
M	3-[(Bromo-sulpho-8-amino-5-hydroxy-4-imino-1-oxo-1,4-dihydronaphthalenyl)amino] -N,N,N-trimethylbenzeneaminium chloride		Impurity	1		495/497	634,590 (sh)	C19H20BrN4O5S
N	3-[(Bromo-sulpho-8-amino-5-hydroxy-1,4-dioxo-1,4-dihydronaphthalenyl)amino] -N,N,N-trimethylbenzeneaminium chloride		Impurity	1		496/498	568	C19H19BrN3O6S
O	3-[(Sulpho-8-amino-5-hydroxy-4-imino-1-oxo-1,4-dihydronaphthalenyl)amino] -N,N,N-trimethylbenzeneaminium chloride		Subsidiary colour	1		417	634, 590 (sh)	C19H21N4O5S
P	3-[(Chloro-sulpho-8-amino-5-hydroxy-4-imino-1-oxo-1,4-dihydronaphthalenyl)amino] -N,N,N-trimethylbenzeneaminium chloride		Impurity	1		451	574	C19H20ClN4O5S

SCCS comment

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?

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5. MINORITY OPINION

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6. REFERENCES

1. Meinigke, B. (2006). Raw Material Specification – C 059. Henkel KGaA, Düsseldorf, Report No. R 0600026
2. Kynoch, S.R. (1986). Acute Oral Toxicity to Rats of Arianor Steel Blue. Huntingdon Research Centre Ltd., Cambridgeshire/UK, internal study code: 85131D/WLH 4/AC. Archive code at Henkel KGaA, Düsseldorf, Report No. R 9501207
3. Arcelin G. (2004). C 059: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application). RCC Ltd, Itingen, Switzerland, internal study code: 853986. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0400811
4. Arcelin, G. (2004). C 059: Primary Eye Irritation Study in Rabbits. RCC Ltd, Itingen,Switzerland, internal study code: 853987. Archive code at Henkel KGaA, Düsseldorf,Report No. R 0400801
5. Ullmann, W. (2001). Arianor Steel Blue: Local Lymph Node Assay (LLNA) in Mice (Identification of Contact Allergens). RCC Ltd, Itingen, Switzerland, internal study code: 795363. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0100254
6. 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. R0300002
7. Bachmann, M., Flade, D. and Krinke, G. (2006). C 059 – 90-Day Oral (Gavage) Toxicity Study in Wistar Rats Followed by a 4 Week Recovery Period. RCC Ltd, Itingen, Switzerland, internal study number: A16470. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0600019(Vol. I and II)
8. Gerspach, R. and Flade, D. (2006). C 059 – Prenatal Developmental Toxicity Study in the Han Wistar Rat. Research Toxicology Centre (RTC) Rom/I, RCC Ltd, Itingen, Switzerland, internal study number: A16481. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0600021
9. Krätzer, F. (2005). Bacterial Reverse Mutation Test (Ames Test) with C 059.GenPharmTox BioTech AG Cell Culture & Toxicology, Martinsried, Germany, internal study code 100128. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500198
10. Wollny, H.-E. (2005). Cell Mutation Assay at the Thymidine Kinase Locus (TK+/-) in Mouse Lymphoma L5178Y Cells with C 059, CCC Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 897002, Archive code at Henkel KGaA, Report No. R 0500401
11. Krätzer, F. (2005). In vitro micronucleus test with C 059, GenPharmTox BioTech AG, Martinsried, Germany, internal study code 100135. Archive code at Henkel KGaA, Report No. R 0500310

12. Honarvar, N. (2005). Micronucleus Assay in Bone Marrow Cells of the Mouse with C059. RCC Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 909502. Archive code at Henkel KGaA, Düsseldorf, Report No. R 0500437
13. Honarvar, N. (2003). In vivo/in vitro Unscheduled DNA synthesis in rat hepatocytes with Basic Blue 99 (C 059). RCC Cytotest Cell Research GmbH, Rossdorf, Germany, internal study code 792600. Archive code at Henkel KGaA, Düsseldorf, Report No. R0300571

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*

Batch No	HPLC-PDA data
Batch RS2798801	**Detector PDA L-2450** 1: 250 nm, 4 nm Results

Pk #	Name	Retention Time	Area	Area Percent
1	A1	2,807	76469	0,109
2	A2	3,387	373216	0,530
3	M1	17,573	62093	0,088
4	M2	20,060	493322	0,701
5	D2	20,787	845987	1,201
6	D3	21,327	25809798	36,655
7	T2	21,640	223205	0,317
8	D5	21,867	213419	0,303
9	M2	22,200	716968	1,018
10	D5	22,747	362206	0,514
11	T2	22,960	2940571	4,176
12	D4	23,347	2846759	4,043
13	T3	23,547	1259885	1,789
14	T3	24,247	2926186	4,156
15	T2	24,787	930366	1,321
16	D5	25,047	2140861	3,040
17	D4	25,620	10849541	15,408
18		26,767	2139409	3,038
19	D4	27,307	4702229	6,678
20		27,647	812561	1,154
21	T5	28,140	1275192	1,811
22		28,373	53318	0,076
23		28,760	444057	0,631
24	T5	29,313	4458174	6,331
25	N1	29,740	1580425	2,244
26	T6	30,693	56413	0,080
27	T7	31,813	684980	0,973
28	T5	32,700	429202	0,610
29		33,200	399826	0,568
30		33,973	139394	0,198
31		37,713	84265	0,120
32		38,680	83027	0,118
Totals			70413324	100,000

Batch 74/75

1: 250 nm, 4 nm
Results

Pk #	Name	Retention Time	Area	Area Percent
1	A1	2,700	561285	0,393
2	A2	3,460	2776670	1,945
3	A3	4,860	1607192	1,126
4	D1	8,447	1407895	0,986
5	A4	9,820	388356	0,272
6		12,467	491966	0,345
7	M1	17,773	3403664	2,384
8	M2	18,553	386585	0,271
9	M2	20,180	453992	0,318
10	D2	20,740	6641408	4,652
11	D3	21,260	70402380	49,316
12	T1	21,887	1654799	1,159
13	M2	22,067	1408617	0,987
14	T2	22,233	2336724	1,637
15		22,907	222682	0,156
16	T3	23,347	2506664	1,756
17	T4	23,560	1521554	1,066
18	T3	24,073	15483505	10,846
19	D4	25,187	3635854	2,547
20	D5	25,967	1778877	1,246
21	D4	26,733	15574076	10,910
22	T5	28,893	1942774	1,361
23	N1	31,727	3601472	2,523
24		35,900	266817	0,187
25		41,313	1383596	0,969
26		45,253	344468	0,241
27		49,700	572388	0,401
Totals			142756260	100,000

**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

Batch RS2798801			Batch 74/75		
Component/ Name	Peak No. of all isomers	Area percent of all isomers	Component/ Name	Peak No. of all isomers	Area percent of all isomers
D3	6	36.7%	D3	11	49.3%
D4	12, 17, 19	4.0+15.4+6.7= 26.1%	D4	19, 21	2.6+10.9 = 13.5%
D5	8, 10, 16	0.3+0.5+3.0 = 3.8%	D5	20	1.3%
T2	7, 11, 15	0.3+4.2+1.3 = 5.8%	T2	14	1.6%
T3	13, 14	1.8+4.2 = 6.0%	T3	16, 18	1.8+10.9 = 12.7%
T5	21, 24, 28	1.2+6.3+0.6 = 8.1%	T5	22	1.4%

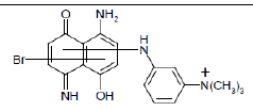
SCCS general comments on Purity: Basic Blue 99 is a mixture of 23-32 substances of varying concentrations as demonstrated by the HPLC analysis of two batches RS2798801 and 74/75 (Table 1). The SCCS is not convinced that all components of Basic Blue 99 (batches RS2798801 and 74/75) are adequately characterised 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

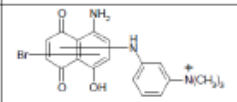
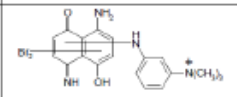
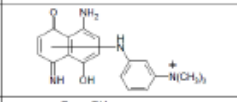
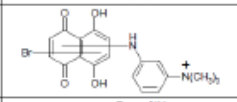
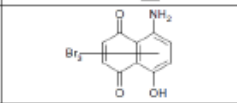
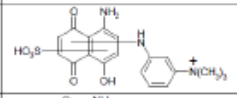
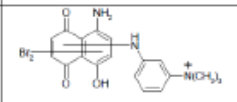
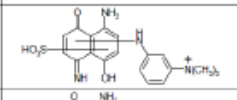
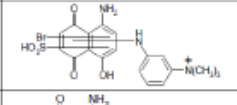
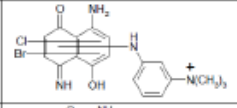
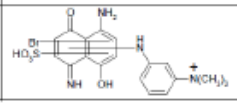
Basic Blue 99 is a sum of 3 isomers with 3-[(4-amino-6-bromo-5,8-dihydro-1-hydroxy-8-imino-5-oxo-2-naphtyl)amino]-N,N,N trimethylanilinium chloride as main isomer, according to ^{1}H-NMR. Purity (% HPLC): >48 area-% (Table 1)

Table 1: Analytical description of Batches used in Toxicity studies or actual market materials

ID	Structure	MW	Peak no.	Range (area%)	74/75 (area-%)	RS27988101 (area-%)	125 (area-%)	140 (area-%)	106106 (area-%)	107664 (area-%)
Main		415 / 417	10, 13, 14	>48	62.8	50.2	48.2	57.3	64.1	67.8

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)

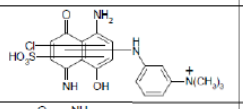
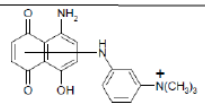
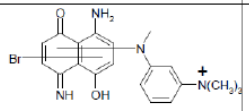
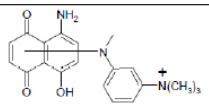
Table 2: Subsidiary colours

ID	Structure	MW	Peak no.	Range (area%)	74/75 (area-%)	RS27988101 (area-%)	125 (area-%)	140 (area-%)	106106 (area-%)	107664 (area-%)
F		416/ 418	15, 16, 23, 24, 25, 26, 27, 28	≤26.5	8.4	16.9	26.0	19.6	7.0	7.3
E		493 / 495 / 497	18, 20 22	≤15	11.8	14.6	4.4	5.5	7.4	10.8
A		337	1, 5	≤9.5	1.7	---	9.3	3.7	1.6	0.9
J		417 / 419	30	≤6.0	4.1	4.8	2.0	3.4	5.4	5.8
L		423-429	39, 40	≤5.0	2.60	1.3	1.7	1.5	4.4	4.3
B		418	4, 11	≤4.0	1.80	2.0	2.8	2.2	3.8	---
G		494 / 496 / 498	31, 32, 34	≤3.0	2.10	2.8	1.2	0.8	2.7	2.0
O		417	3	≤3.0	1.0	---	2.0	2.6	---	---
N		496 / 498	12	≤2.5	0.7	2.1	0.3	---	---	---
X		449 / 451 / 453	21	≤1.7	---	1.7	---	---	---	---
M		495 / 497	7	≤1.6	0.9	---	---	1.3	---	---

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

ID	Structure	MW	Peak no.	Range (area %)	74/75 (area-%)	RS27988101 (area-%)	125 (area-%)	140 (area-%)	106106 (area-%)	107664 (area-%)
P		451	6	≤1.3	---	---	1.3	0.2	0.6	--
C		338	8	≤1.2	0.4	0.6	---	1.2	0.2	0.5
K		430 / 432	29	≤0.9	0.9	---	---	0.4	---	---
Q		352	17	≤0.5	0.2	0.5	0.1	---	0.2	---
---	Not known	?	38	≤1.4	1.4	---	---	---	0.6	0.5
---	Not known	-	37	≤0.9	--	0.9	---	---	0.3	---

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)

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

*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.