Scientific Committee on Consumer Safety

SCCS

OPINION ON

HC Blue 17

COLIPA n° C184

The SCCS adopted this opinion at its 18th plenary meeting

of 26 February 2013

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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1. BACKGROUND

The hair dyeing ingredient HC Blue 17 CAS n.16517-75-2 (C184) is an ingredient, which is newly introduced on the EU market. The responsible company is submitting a safety dossier for review by the Scientific Committee on Consumer Safety. Industry requests use of this ingredient in oxidative and non–oxidative colouring products with a concentration on head of maximum of 2.0%. Submission I, which has been received in September 2011, presents the scientific data on the above mentioned substance.

2. TERMS OF REFERENCE

1. *Does the SCCS consider HC Blue 17 safe for use as an oxidative and a non-oxidative hair dye with a concentration on-head of maximum 2.0% taken into account the scientific data provided?*

2. *And/or does the SCCS recommend any further restrictions with regard to the use of* HC Blue 17 *in any hair dye formulations?*

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1. Chemical identity

HC Blue 17 (INCI name)

3.1.1.1. Primary name and/or INCI name

HC Blue 17 (INCI-name)

Comment
In certificate of analysis of batch XB20178, INCI name is reported as Cationic Blue 347

3.1.1.2. Chemical names

3-[(4-amino-3-methyl-9,10-dioxo-9,10-dihydroanthracen-1-yl)amino]-N,N,N-trimethylpropan-1-aminium, methylsulfate salt

[3-(4-Amino-3-methyl-9,10-dioxo-9,10-dihydro-anthracen-1-ylamino)-propyl]-trimethyl-ammonium methylsulfate (CAS name)

Ammonium, [3-[(4-amino-3-methyl-1-anthraquinonyl)amino]propyl]trimethyl-, methyl sulphate (ECHA pre-registered substance)

Ammonium, [3-[(4-amino-3-methyl-1-anthraquinonyl)amino]propyl]trimethyl-, methyl sulfate (8CI) (ECHA pre-registered substance)

3.1.1.3. Trade names and abbreviations

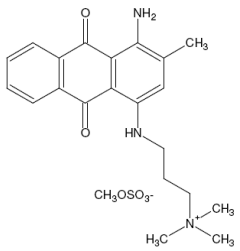
Cationic Blue 347

Blue 347

3.1.1.4. CAS / EC number

CAS: 16517-75-2
EC: 605-392-2 (list number in the EC format as a pre-registered substance)

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{22}H_{29}N_3O_6S$

3.1.2. Physical form

Blue Powder

3.1.3. Molecular weight

Molecular weight: 463 g/mol

3.1.4. Purity, composition and substance codes

Four batches of HC Blue 17 were used for the toxicity studies: DYBB0847, DYBB0539, DYBB0657 and XB20178 as described in the following table

Batches used in respective toxicological studies

DYBB0847	DYBB0539	DYBB0847 XB 20178	DYBB0657	XB20178
Eye irritation Skin irritation Skin Permeability 14 day oral toxicity Ames Test Cell Mutation Assay at HPRT Locus	Local Lymph Node Assay (LLNA) Cell Mutation Assay at Micronucleus Test at TK+/-locus Micronucleus assay in vitro	Subchronic toxicity	Micronucleus Test in vivo	Teratogenicity

Chemical characterisation of HC Blue 17

- Batch XB020178 was chemically characterised by NMR, and IR spectroscopy.
- It is reported that the batch DYBB0847 was characterised by NMR, but report on the NMR analysis was not provided. The UV-Vis spectrum of batch DYBB0847 was identical with the UV-Vis spectrum of the batch XB20178.

- No data was submitted on the chemical characterisation of HC Blue 17 in the batches DYBB0657 and DYBB0539.

Purity of HC Blue 17

- HPLC purity of HC Blue 17 of batch DYBB0847 in various tests has been described as 95%, 97%, 93.3% (HPLC, %peak area). According to the analytical report the content of HC Blue 17 in this batch has been **estimated** to be 93.3% (HPLC, %peak area). In the Summary Submission, using a different HPLC method, the HC Blue 17 content in this batch was reported to be 93.1% (HPLC, %peak area).
- HPLC purity of HC Blue 17 of batch XB20178 in two different tests has been described as 37% and 97% (HPLC, %peak area). According to the analytical report the content of HC Blue 17 in this batch has been **estimated** to be 36.4% (HPLC, %peak area). In the Summary Submission, using a different HPLC method, the HC Blue 17 content in this batch was reported to be 93.1% (HPLC, %peak area).
- In reference 9 the content of HC Blue 17 in the batch DYBB0657 is reported as 100%. In the Summary Submission, the HC Blue 17 content in this batch is described as 94.4% (HPLC, %peak area). However, no study is submitted to demonstrate any of these values.
- In the studies performed with the batch DYBB0539, the content of HC Blue 17 is reported as 98.5%, (HPLC, %peak area). In the Summary submission the content of HC Blue 17 in this batch is described as 93.9% (HPLC, %peak area), However, no study is submitted to demonstrate any of these values.
- HC Blue 17 is a secondary amine, and thus is prone to nitrosation and formation of nitrosamines. It should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb.

3.1.5. Impurities / accompanying contaminants

The impurities determined in the batches DYBB0847 and XB20178 of HC Blue 17 were as follows:

Batch DYBB0847
HPLC peak area at 254 nm

Trimethyl-[3-(3-methyl-4-methylamino-9,10-dioxo-9,10-dihydro-anthracen-1-ylamino)-propyl]-ammonium methylsulfate, (N-methyl HC Blue 17):	1.8%
1-Amino-2-methyl-anthra-9,10-quinone:	0.2%
2-Methyl-anthraquinone:	0.09%
Two unknowns:	0.33% and 0.29% respectively

Metals
Fe, Cu, Pb, Ni and Cd: 100 ppm, 830 ppm, <1 ppm, 3.6 ppm, and <0.5 ppm respectively

Batch XB20178
HPLC peak area at 254 nm

Trimethyl-[3-(3-methyl-4-methylamino-9,10-dioxo-9,10-dihydro-anthracen-1-ylamino)-propyl]-ammonium methylsulfate, (N-methyl HC Blue 17) :	1.8%
Two unknowns:	0.75% and 0.04% respectively

Metals

Na, Fe, Cu, Pb, Ni, Cd and Hg: 0.15% (w/w), 106 ppm, 390 ppm, <2 ppm, 1.8 ppm, <0.5 ppm and <2 ppm, respectively

Chlorobenzene and o-dichlorobenzene: < 0.025% (250 ppm, limit of detection)

Ref.: 11

Purity and impurities of HC Blue 17 reported in Summary Submission, the HPLC method was different than used in Ref. 11.

Purity/impurity (% HPLC peak area)	Batch 0539	Batch 0657	Batch DYBB0847 and Batch XB20178
Purity HC Blue 17	93.9 %	94.4 %	93.1 %
1,4-Diamino-2-methyl-anthra-9,10-quinone	0.4 %	0.7 %	3.7
1-Amino-2-methyl-anthra-9,10-quinone	0.8 %	0.2 %	/
Unknown 1, % HPLC peak area	0.2 %	0.1 %	1%
Unknown 2, % HPLC peak area	/	/	0.2%
Unknown 3, % HPLC peak area	/	0.8 %	/
Unknown 4 , % HPLC peak area	/	1.4 %	/

Comment
The organic impurities described in the analytical report of HC Blue 17 batches DYBB0847 and XB20178 (ref. 11) are qualitatively/quantitatively different than those reported in Summary submission. There was no study report to demonstrate the reported impurities in the batches DyBB0539 and DYBB0657 of HC Blue 17.
The method of analysis of chlorobenzene and dichlorobenzene is not sensitive enough as the limit of detection (250 ppm) is too high.
The mass balance of the identified substances is not 100% in any of the batches.

3.1.6. Solubility

Water: > 40%
DMSO: > 10%

Comment
No report is submitted for solubility testing. Water solubility is not determined by EU Method A.6

3.1.7. Partition coefficient (Log Pow)

Log P_{ow}: /

3.1.8. Additional physical and chemical specifications

Melting point: /
Boiling point: /
Flash point: /
Vapour pressure: /
Density: /
Viscosity: /
pKa: /
Refractive index: /
UV_Vis spectrum (200-800 nm): λmax 258 nm, 562 nm, 604 nm

3.1.9. Homogeneity and Stability

The test solutions used for 90 day oral toxicity study and prenatal developmental study were shown to be homogeneous – A maximum variation of 2.1% was found in the HC Blue 17 content in top, middle and bottom of the test solutions

HC Blue 17 in the test solutions used for 90 day oral toxicity study and prenatal developmental study was shown to be stable for 7 days at room temperature – A maximum variation of 10.2% was found in the HC Blue 17 content in the test solutions after 7 days storage period.

Ref.: 11, 12

The stability of HC Blue 17 in oxidative environment was determined by mixing (1:1) 1% Blue 347 solution in MEA buffer pH 10 with 6% aqueous peroxide. The UV-Vis absorption spectra of this mixture at time 0 and after 30 minutes were shown to be overlapping. Blue 347 is therefore considered stable in an oxidative environment

In the Ames test, it is described that HC Blue 17 is stable in DMSO solutions for at least up to 96 hours, but no documentation was provided

In *in vitro* dermal absorption study, HC Blue 17 was described to be stable in the test formulation up to one year, but no documentation was provided.

General Comments on physico-chemical characterisation

- Characterisation of HC Blue 17 in two of the 4 batches was not performed.
- Purity of HC Blue 17 is not determined adequately (varying results, %peak area HPLC and no reference standard used) in any of the four batches, and no study was submitted to demonstrate the reported purity of HC Blue 17 in two batches DYBB0539 and DYBB0657**.**
- The impurities in two batches of HC Blue 17 reported in Summary Submission are qualitatively/quantitatively different than those found by an earlier analysis. There was no study report to document the impurities, in all batches of HC Blue 17, described in the Summary Submission.
- Log Pow of HC Blue 17 has not been measured.
- Water solubility of HC Blue 17 has not been determined by EU method.
- No additional physico-chemical properties such as melting point, density, etc. of HC Blue 17 have been reported.
- Stability of HC Blue 17 in typical hair dye formulation has not been documented.

3.2 Function and uses

HC Blue 17 is used as a hair colouring agent up to 2% on-head concentration of in non-oxidative as well as in oxidative hair dye formulations.

3.3 Toxicological Evaluation

3.3.1 Acute toxicity

No study on acute toxicity was provided. The following details were taken from reference 9, micronucleus test.

In the pre-test the mice treated i.p. with 100 mg/kg bw died within 1 h after treatment. After treatment with 50 mg/kg bw, 1 mouse died after 6 h. The surviving mice showed reduction in spontaneous activity, abdominal position and ruffled fur at least the first 6 h after treatment. At the lower doses these clinical effects were less obvious. Mice treated with doses of 25 mg/kg bw and above had blue coloured urine.

3.3.1.1 Acute oral toxicity

No data submitted

3.3.1.2 Acute dermal toxicity

No data submitted

3.3.1.3 Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Guideline:	OECD 404 (2002)
Species/strain:	New Zealand White rabbit, SPF
Group size:	3 (1 male, 2 females)
Test substance:	Blue 347
Batch:	DYBB0847
Purity:	97%
Vehicle:	purified water
Dose:	0.5 g, moistened with 0.5 ml of purified water
Application:	topical 4 hours, semi-occlusive
GLP:	in compliance
Study period:	16 January – 4 February 2008

Blue 347 was applied by topical semi-occlusive application of 0.5 g to the clipped flank of each of three young adult New Zealand White rabbits. 0.5 g of Blue 347, moistened with 0.5 ml of purified water was placed on a surgical gauze patch and applied to the intact skin of the clipped area. The patch was covered with a semi-occlusive dressing and this was wrapped around the abdomen and anchored with tape. The duration of the treatment was four hours. The scoring of skin reactions was performed 1, 24, 48 and 72 hours, as well as 7, 10 and 14 days after removing of the dressing.

Results
No skin reactions were seen at the application site of any animals at any of the observation times. The test item caused a slightly blue staining of the treated skin 1 hour after test item exposure up to day 14.

Conclusions
Neat Blue 347 is not irritant to rabbit skin under the conditions of this experiment.

Ref.: 2

Comment
It is not stated whether the blue staining hindered readings.

3.3.2.2 Mucous membrane irritation

Guideline:	OECD 405 (2002)
Species/strain:	New Zealand White rabbit, SPF
Group size:	3 (1 male, 2 females)
Test substance:	Blue 347
Batch:	DYBB0847
Purity:	97%
Vehicle:	/
Dose:	0.1 g
GLP:	in compliance
Study period:	4 – 26 February 2008

The primary eye irritation of Blue 347 was investigated by instillation of 0.1g of the test item into the left eye of each of three young adult New Zealand White rabbits. Scoring of irritation effects was performed at 1, 24, 48 and 72 hours, as well as 7, 10 and 14 days after instillation.

Results
The instillation of Blue 347 into the eye resulted in mild, early-onset and transient ocular changes, such as corneal opacity, reddening of the conjunctivae and sclera, discharge and chemosis one hour after treatment. These effects were reversible and were no longer evident 24, 48 and 72 hours after treatment. Two animals showed slightly redness of conjunctivae 24 hours after treatment. No abnormal findings were observed in the iris of any animal. No corrosion was observed at any of the measuring intervals. A slight to marked blue staining of the treated eyes caused by the test item was observed in all animals from 1 hour reading up to day 7 or 10.

Ref.: 1

Comment
Neat Blue 347 causes slight and reversible irritation to rabbit eyes.

3.3.3 Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429 (2002)
Species/strain:	Mice, CBA/CaOlaHsd
Group size:	4 females per test group
Test substance:	Blue 347
Batch:	DYBB0539
Purity:	98.5% (HPLC)
Vehicle:	DMSO
Concentration:	0, 2.5, 5, 10, 15% (w/v)
Positive control:	α-Hexylcinnamaldehyde in acetone:olive oil (4:1)
GLP:	in compliance
Study period:	16 February – 7 March 2006

The study of the possible allergic potential of Blue 347 was done by the local lymph node assay. Four groups of four female mice were treated with 2.5, 5, 10 and 15% of Blue 347 in DMSO by topical application at the dorsum of each ear lobe on three consecutive days. A control group was treated with the vehicle only. Five days after the first topical application, the mice were intravenously injected with radio-labelled thymidine. Animals were sacrificed and the draining auricular lymph nodes excised. The proliferative capacity of pooled lymph node cells were determined by the incorporation of ^{3}H-methyl thymidine measured by β–scintillation counter.

Results

Treatment	Concentration	Stimulation index
Blue 347 in DMSO	2.5% 5% 10% 15%	0.97 1.11 1.54 1.44
α-Hexylcinnamaldehyde in acetone:olive oil (4:1)	5% 10% 25%	6.08 13.59 26.41

The EC3 could not be calculated, since all SI's are below 3.

Conclusions
The test item Blue347 was found not to be a skin sensitizer under the described conditions.

In the report, the rationale for using a maximum concentration of 15% in DMSO was stated as:
"To determine the highest non-irritant test concentration or the highest technically applicable concentration, two non-GLP pretests were performed in two mice (pretests excluded from Statement of Compliance). The data showed that the highest test item concentration, which could be technically used, was a 25% dilution in ethanol:deionised water (7+3). At this concentration, the treated mouse did not show any signs of irritation. Since the sponsor requested that DMSO should be used as vehicle, a second pretest was performed. The data showed that the highest test item concentration, which could be technically used was a 15% dilution. At this concentration, the treated mouse did not show any signs of irritation."

Ref.: 3

Comment
The data in the rational statement were not provided.
The highest concentration tested (15%) in the LLNA was considered too low. A sensitisation potential of Blue 347 cannot be excluded.

3.3.4 Dermal / percutaneous absorption

Guideline:	OECD 428 (2004)
Tissue:	pig skin
Membranes:	dermatomed pig skin (ear), 400 ± 80 µm
Skin integrity:	conductivity (< 900 µS/cm)
Method:	flow-through diffusion chambers, 1 cm²
Replicate cells:	12 replicates (6 donors) per formulation type
Test substance:	Blue 347
Batch:	DYBB0847
Purity:	95% (HPLC)
Formulation:	Color formulation 2% Blue 347 (non-oxidative) Color formulation 4% Blue 347, mixed 1:1 with Topchic Lotion 6% (oxidative preparation)

Dose applied: 20 µL/cm²
Receptor fluid: saline (0.9% NaCl in water)
Solubility receptor fluid: ≥ 1 µg/mL
Stability: /
Analytical method: HPLC
GLP: in compliance
Study period: 15 – 31 July 2009

The dermal penetration of Blue 347 from two different formulations (non oxidative and oxidative) containing the same final concentration of Blue 347 (2%) was investigated in dermatomed pig ear skin from 6 different donors.
The test item was applied on each skin sample at an amount of 20 µL/cm² (corresponding to about 400 µg/cm² Blue 347 each) for 0.5 hours and then washed off using deionised water and 10% shampoo solution.
The receptor solution was slowly pumped through receptor chambers with a flow rate from 0.8 to 1.1 mL per hour and fractionated 0.5, 2, 4, 8, 12, 16, 20, 23 and 24 hours following application.
The stratum corneum was separated by tape stripping from the remaining skin.
Analysis for the presence of Blue 347 was carried out by means of HPLC-DAD.

Results
Under the reported conditions 1.76 ± 2.96 µg/cm² (0.637 ± 1.056% of applied dose) of Blue 347 under non oxidative conditions and 2.18 ± 2.05 µg/cm² (0.883 ± 0.834 % of applied dose) of Blue 3347 under oxidative conditions were systemic bioavailable.

Non-oxidative conditions

	Color formulation 2 % Blue 347					
Amount of Blue 347 in	Expressed as µg/cm² of skin surface mean ± S.D. (n = 12)#			Expressed as % of dose mean ± S.D. (n = 12)#		
Amount applied	271	±	9.77	100	±	3.46
Penetration into the receptor fluid after 24 hours	0.286	±	0.418	0.1042	±	0.149
Stratum corneum (isolated by stripping, after 24 hours)	0.454	±	0.303	0.1671	±	0.111
Remaining Epidermis + Dermis (after 24 hours)	1.47	±	2.89	0.533	±	1.03
Washing solution (after 0.5 hours)+ Cap fraction and SN solution (after 24 hours)	271	±	13	100.1	±	2.85
Recovery	273	±	13.4	100.9	±	2.58
Total absorption (receptor fluid + epidermis + dermis excluding tape strip)	1.76	±	2.96	0.637	±	1.056

only valid values with a recovery of >85% were used
The supernatant of impedance measurement after 24h is named SN solution

Oxidative conditions

	Color formulation 4 % Blue 347 to be mixed 1:1 with Topchic Lotion 6 %					
Amount of Blue 347 in	Expressed as µg/cm^2 of skin surface mean ± S.D. (n = 11)#			Expressed as % of dose mean ± S.D. (n = 11)#		
Amount applied	248	±	19.7	100	±	7.60
Penetration into the receptor fluid after 24 hours	0.893	±	1.35	0.354	±	0.509
Stratum corneum (isolated by stripping, after 24 hours)	0.787	±	0.539	0.3219	±	0.234
Remaining Epidermis + Dermis (after 24 hours)	1.28	±	1.16	0.529	±	0.510
Washing solution (after 0.5 hours)+ Cap fraction and SN solution (after 24 hours)	239	±	10.3	96.9	±	4.81
Recovery	242	±	10.4	98.1	±	4.92
Total absorption (receptor fluid + epidermis + dermis excluding tape strip)	2.18	±	2.05	0.883	±	0.834

only valid values with a recovery of >85% were used.
The supernatant of impedance measurement after 24 h is named SN solution.

Ref.: 4

Comment
These were well performed studies.
The mean + SD (1.76 + 2.96 = 4.72 µg/cm²) in non-oxidative conditions and (2.18 + 2.05 = 4.23 µg/cm²) in oxidative conditions may be used to calculate MoS.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (14 days) oral toxicity

14-Day range-finding study

Guideline: /
Species/strain: rat, SPF-bred Wistar
Group size: 5 males and 5 females per dose group
Test substance: Blue 347
Batch: DYBB0847
Purity: 93.1%
Vehicle: bidistilled water
Dose levels: 0, 100, 300, 1000 mg/kg bw
Dose volume: /
Route: oral
Administration: 14 days
GLP: /
Study period: 2008

40 rats (20 males and 20 females, strain HanIbm:WIST, SPF) were used for this assay. The body weights of the animals were approximately 190 g (±20%) for males and 150 g

(±20%) for females at the beginning of the study. The test material was homogenized in bi-distilled water and was prepared daily before administration. Analysis of homogeneity and stability of Blue 347 in bi-distilled water was performed and proven before administration to the animals. The following dose levels were tested in this study: 0, 100, 300 and 1000 mg/kg bw/d. The animals (5 males, 5 females, each concentration) were treated by oral gavage, once daily, for 14 days. Clinical signs, food consumption and body weights were recorded periodically during pretest, and treatment period. At the end of the treatment period all animals were killed, necropsied and examined post mortem.

Results

	100 mg/kg bw/d	**300 mg/kg bw/d**	**1000 mg/kg bw/d**
Viability/mortality	All animal survived	All animals survived	All animals survived
General Cageside observations	Discoloured faeces	Discoloured faeces	Discoloured faeces
Macroscopic findings	No test item related changes	No test item related changes	No test item related changes

On the basis of the results obtained in the 14-day dose range finding study the following dose levels for the 90 day subchronic study were proposed: 0, 40, 200 and 1000 mg/kg bw/d.

Ref.: 10

Comment of the SCCS
The study was not provided in detail.

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

Guideline:	OECD 408 (1998)
Species/strain:	rat, HanRcc: WIST (SPF)
Group size:	10 males and 10 females per dose group 5 males and 5 females (recovery group: 0 and 1000 mg/kg bw)
Test substance:	Blue 347
Batch:	DYBB0847 (from 20 May to 21 July 2008) XB20178 content 36.4% pure dye (from 22 July 2008 to end of treatment)
Purity:	93.1%
Vehicle:	bidistilled water
Dose levels:	0, 40, 200, 1000 mg/kg bw/d
Dose volume:	
Route:	oral (gavage)
Administration:	daily
GLP:	in compliance
Study period:	13 May 2008 – 2 September 2009

Note of the study authors
The initial delivery of the test item proved to be insufficient to complete the study and additional test item was requested from the sponsor. Both deliveries used in this study came from the same batch. The second shipment consisted of the original solid test item dissolved in water to a concentration of 37%. The dose formulation procedure was adapted accordingly.

Blue 347 was homogenized in bi-distilled water. Analysis of homogeneity and stability in bidistilled water was performed before administration to the animals. The individual

concentrations varied in the range of 81.7% to 108.7% of the nominal concentration and were found to be homogeneously distributed in the vehicle. The test item was also found to be stable in the vehicle for at least seven days when stored at room temperature. Therefore, the mixture of the test article and vehicle were prepared weekly. Concentration, homogeneity and stability (after 4 h and 7 d) of the dose formulation were determined in samples taken after experimental start.

The animals (10 males, 10 females at each dose group) were treated by oral gavage, once daily for at least 13 weeks at the doses 0, 40, 200, 1000 mg/kg bw/d. Control animals were treated with vehicle only. 5 animals per sex of the control group and high dose group were allowed a 4-week treatment-free recovery period.

Clinical signs, outside cage observation, food consumption and body weights were recorded periodically during pretest, the treatment and recovery periods. Functional observation battery was performed during the last week of treatment. All animals were killed, necropsied and examined post mortem. The clinical laboratory investigation including haematology, clinical biochemistry, urinalysis and histological examinations on organs and tissues from all animals was performed. Ophthalmoscopy was mistakenly omitted from the study.

Results
At 1000 mg/kg bw/d three rats were found dead before scheduled necropsy. At 200 and 40 mg/kg bw/d one male of each group was found dead. Congestion of the lung, alveolar haemorrhage and/or oedema was noted and the cause of the deaths was considered to be respiratory failure due to aspiration of the formulation or due to gavage error. Bluish discoloration of the faeces was noted at all dose levels. There were no test item-related changes during functional observational battery (grip strength measurement, locomotor activity). Food consumption and body weights were similar to that of controls.

Ophthalmoscopy was not performed. Haematology, clinical biochemistry and urinalysis values were in the common range of historical controls and all deviations were considered incidental. No test item-related changes in organ weights were recorded. Intestinal lesions were found at 1000 mg/kg bw/d (increased histiocytes in lamina propria and hyaline inclusions in the epithelium of duodenum, jejunum, ileum, cecum, colon or rectum of males and females). After recovery these lesions were not recorded.
In addition, at 1000 as well as at 400 mg/kg bw/d respiratory metaplasia of olfactory epithelium, hyperosteosis of the turbinates, submucosal mineralization and inflammatory exudates were noted. At 40 mg/kg bw/d respiratory metaplasia of olfactory epithelium and inflammatory exudates were observed in 2 females. These findings were considered to result from deposition of minute amounts of dosing solution that remained at the laryngopharynx junction following withdrawal of the ball-tip intubation tube and that subsequently migrated into the nasal turbinates as the animal exhaled. The applicant argued that such findings in an oral toxicity study performed by oral gavage are due to physical/chemical properties of the administered test substance and not due to an intrinsic toxicity; therefore, such procedure-related findings should be considered to be a local effect related to the mode of administration (oral gavage) and not be used for a risk assessment following human skin contact with the substance.

Ref.: 11

Pathology peer review
In an external expert evaluation the results and interpretation of the 90-day oral toxicity (gavage) study in the Wistar rat with Blue 347 as well as the toxicology summary prepared by the applicant for submission to the Scientific Committee on Consumer Safety (SCCS) were assessed.

The main difference in interpretation between the study pathologist and the reviewing pathologist involved the diagnosis of respiratory metaplasia of the olfactory epithelium at

level III, and to a lesser extent level IV. Although there was some noticeable variability of sectioning among animals, most sections from the level III corresponded to levels 11 or 12 and most sections from level IV corresponded to levels 23 through 25, as described by Mery *et al.* (1994). These levels have normal respiratory epithelium in the dorso-lateral meatus (Level III) or ventral meatus (Level IV). Based on the absence of other significant changes, the reviewing pathologist assumed that this normal respiratory epithelium was interpreted as metaplastic olfactory epithelium by the study pathologist. Both the distribution pattern and the nonspecific nature of the changes are consistent with the local deposition of an irritant substance.

As stated in the final study report, the reviewing pathologist agreed that these degenerative changes of the nasal cavity were secondary to the local deposition of the test article following oral gavage procedure. This opinion was based on the distribution of the lesion, the unspecific nature of the lesion (no single cell type seemed to be more sensitive than another), and the lack of a clear dose response between the 200 and 1000 mg/kg bw/day dose levels. The higher incidence of the findings at these dose levels compared to 40 mg/kg bw/day could be related to increased struggling by the dosed animals that made dosing more difficult. This increased struggling could be due to the higher concentration of a test article that may have had bad taste and/or was more irritant to the respiratory tract at higher concentrations.

The consulting pathologist is in agreement with the interpretation of the study authors that there was no evidence of any adverse test article-related effect on mortality and/or respiratory tract pathology:
The No-Observed Adverse Effect Level (NOAEL) was 1000 mg/kg/day based on the absence of any adverse test article-related findings. The 5 unscheduled deaths that occurred during the course of the study were not test article related and were either related to gavage error or an undetermined cause. The various degenerative changes noted in the nasal cavity (levels III and IV) were considered secondary to the local deposition of the test article during the dosing procedure and are not relevant to any risk assessment for human use of the test article.

Ref.: 22, 23

Comment of the SCCS
The reported irritant effects in the nasal cavity are quite unusual in an oral gavage study and may be due to technical problems during administration of the test substance. HC Blue 17 is not considered a strong mucosal irritant: no signs of stomach irritation were observed and the substance was considered only slightly irritant to the rabbit eyes (see Ref.: 1). The local irritant effects in the nasal cavity were concentration-related and also observed at the lowest dose 40 mg/kg bw/d. No NOEL was derived in this study. However, for the foreseen use as hair dye ingredient these effects are not considered relevant. Due to the intestinal lesions observed at 1000 mg/kg bw/d the NOAEL of systemic toxicity is 200 mg/kg bw/d.

3.3.5.3 Chronic (> 12 months) toxicity

No data submitted

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity in vitro

Bacterial Reverse Mutation Assay

Guideline: OECD 471 (1997)
Species/Strain: *S. typhimurium* TA98, TA100, TA1535, TA1537, and *E. coli* WP2 uvrA
Replicates: triplicate cultures in 2 independent experiments

Test substance:	Blue 347	
Batch:	DYBB0847	
Purity:	97% (HPLC)	
Solvent:	DMSO	
Concentration:	experiment I:	3, 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate without and with S9-mix
	experiment II:	33, 100, 333, 1000, 2500 and 5000 µg/plate without and with S9-mix
Treatment:	experiment I:	direct plate incorporation with 48 h incubation without and with S9-mix
	experiment II:	pre-incubation method with 60 minutes pre-incubation and 48 h incubation without and with S9-mix
GLP:	in compliance	
Study period:	8 September 2008 – 23 September 2008	

Blue 347 was investigated for the induction of gene mutations in *S. typhimurium* and *E. coli* (Ames test). Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the level of toxicity in a pre-experiment for toxicity with all strains both without and with S9-mix. Toxicity was evaluated for 8 concentrations up to the prescribed maximum concentration of 5000 µg/plate on the basis of a reduction in the number of spontaneous revertant colonies and/or clearing of the bacterial background lawn. Experiment I was performed with the direct plate incorporation method, experiment II with the pre-incubation method with 60 min pre-incubation. Negative and positive controls were in accordance with the OECD guideline.

Results
Precipitation of Blue 347 was observed in the overlay agar in the test tubes in experiment I from 1000 µg/plate up to 5000 µg/plate and in experiment II from 2500 µg/plate up to 5000 µg/plate. The undissolved particles of Blue 347 had no influence on the data recording. The plates incubated with Blue 347 showed normal background growth up to 5000 µg/plate without and with S9-mix. Since no toxic effects, evident as a reduction in the number of revertants, were observed in the test groups as well, 5000 µg/plate was chosen as maximum concentration. As in the pre-experiment evaluable plates were obtained for five concentrations or more in the strains used, the pre-experiment is reported as experiment I.
Blue 347 treatments did not result in a biologically relevant increase in the number of revertant colonies in any of the strains tested at any concentration level, neither without nor with S9-mix.

Conclusion
Under the experimental conditions used Blue 347 was not mutagenic in this gene mutation tests in bacteria.

Ref.: 5

In vitro Mammalian Cell Gene Mutation Test (*tk*-locus)

Guideline:	OECD 476 (1997)
Cells:	mouse lymphoma cell line L5178Y ($tk^{+/-}$)
Replicates:	duplicate cultures in a single experiment
Test substance:	Blue 347
Batch:	DYBB 0539
Purity:	98.5% (HPLC)
Solvent:	deionised water
Concentrations:	250, 500, 1000, 2000 and 3000 µg/ml without S9-mix 500, 1000, 2000, 3000 and 4000 µg/ml with S9-mix

Treatment:	4 h treatment both without and with S9-mix; expression period 72 h and a selection period of 10-15 days
GLP:	/
Study period:	7 June 2006 – 3 July 2006

Blue 347 was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-test for toxicity with 4 and 24 h exposures to concentrations up to 4000 µg/ml in the absence and presence of S9-mix measuring suspension growth relative to the concurrent vehicle control cell cultures. In the main tests, cells were treated for 4 h both without and with S9-mix, followed by an expression period of 72 h to fix the DNA damage into a stable *tk* mutation and a selection growth of 10-15 days. Toxicity was measured in the main experiments as percentage suspension and relative total growth of the treated cultures relative to the concurrent vehicle control cell cultures. To discriminate between large (indicative for mutagenic effects) and small colonies (indicative for a clastogenic effect) colony sizing was performed. An increased occurrence of small colonies indicated by a low large/small colonies ratio was associated with clastogenic effects and/or chromosomal aberrations. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-experiment no precipitation visible to the unaided eye occurred. Only in culture 2 without S9-mix the appropriate level of toxicity (about 10-20% survival after the highest concentration) was reached.
Without metabolic activation a biologically relevant increase in the mutant frequency was not found; all mutant values found were within the range of the historical control data.
With metabolic activation discriminating results were found between the 2 cultures per concentration. In culture 1 a dose dependent and statistically significant increase in the mutant frequency outside the range of the negative control data was observed. In culture 2, however, a biological relevant increase in the mutant frequency was not found which was obviously due to a rather high mutant frequency found for the concurrent control cultures. Additionally, the mutant frequency for the positive controls was substantially higher (< 6-fold difference) in culture 2 compared to culture 1. As such the data were interpreted as inconclusive.

Conclusion
Under the experimental conditions used, the outcome of this *in vitro* mammalian cell gene mutation test with Blue 347 was inconclusive.

Ref.: 6

Comment
The SCCS agrees with the conclusion of the authors that the outcome of this *in vitro* mammalian cell gene mutation test is inconclusive since the results of each separate culture per concentration in the presence of S9-mix gave contradictory results.

***In vitro* Mammalian Cell Gene Mutation Test (*hprt* locus)**

Guideline:	OECD 476 (1997)
Cells:	mouse lymphoma cell line L5187Y
Replicates:	duplicate cultures in 2 independent experiments
Test substance:	Cationic Blue 347
Batch:	DYBB0847
Purity:	97% (HPLC, 605 and 254 nm)
Solvent:	culture medium
Concentrations:	experiment I 150, 300, 600, 1200, 1800 µg/ml without S9-mix

		300, 600, 1200, 2400, 3600 µg/ml with S9-mix
	experiment II	75, 150, 300, 600 µg/ml without S9-mix 600, 1200, 2400, 3600, 4800 µg/ml with S9-mix
Treatment:	experiment I	4 h both without and with S9 mix; expression period 6 days and a selection period of 10-15 days
	experiment II	24 h without S9 mix; expression period 6 days and a selection period of 10-15 days 24 h with S9 mix; expression period 6 days and a selection period of 10-15 days
GLP:	in compliance	
Study period:	29 October 2008 - 30 December 2008	

Cationic Blue 347 dissolved in culture medium was assayed for mutations at the *hprt* locus of mouse lymphoma cells both in the absence and presence of metabolic activation. The assay was performed in two independent experiments using duplicate cultures each. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system.
Test concentrations were based on the results of a pre-test for toxicity with 4 and 24 h exposures to concentrations up to 4800 µg/ml, corresponding to the prescribed maximum concentration of 10 mM according to the OECD guideline, in the absence and presence of S9-mix measuring suspension growth relative to the concurrent vehicle control cell cultures. In the main tests, cells were treated for 4 h (both without and with S9-mix) or 24 h (without S9-mix only, experiment II) followed by an expression period of 6 days, to fix the DNA damage into a stable *hprt* mutation and a selection growth of 10-15 days. Toxicity was measured in the main experiments as percentage suspension and relative total growth of the treated cultures relative to the concurrent vehicle control cell cultures. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-test no precipitation occurred at 1200 µg/ml without and with S9-mix; at higher concentrations precipitation could not be evaluated due to the intense colour of Cationic Blue 347. No relevant deviations in pH or osmolarity were observed up to the maximum concentration.
Toxic effects were more prominent in the absence than in the presence of metabolic activation. With S9-mix the appropriate level of toxicity (about 10-20% survival after the highest concentration) was mostly reached, with S9-mix only in experiment II this appropriate level was approached.
In both experiments, a biologically relevant increase in the mutant frequency due to Cationic Blue 347 treatments was not observed at any concentration level, neither without nor with S9-mix.

Conclusion
Under the experimental conditions used, Cationic Blue 347 did not induce mutations at the *hprt* locus of L5178Y mouse lymphoma cells and consequently is not genotoxic (mutagenic) in this gene mutation test.

Ref.: 7

In vitro **Micronucleus Test**

Guideline:	/
Cells:	Chinese hamster V79 cells
Replicates:	duplicate cultures in a single experiment
Test substance:	Blue 347
Batch:	DYBB0539
Purity:	/
Solvent:	deionised water

Concentrations:	36.7, 73.4, 146.9, 293.8, 587.5, 1175, 2350 and 4700 µg/ml without and with S9-mix
Treatment:	24 h treatment without S9-mix, harvest time immediately after the end of treatment 4 h treatment with S9-mix, harvest time 24 h after the start of treatment
GLP:	/
Study period:	19 July 2006 – 4 August 2006

Blue 347 has been investigated in the absence and presence of metabolic activation for the induction of micronuclei in V79 cells. After discussion with the study monitor and with respect to the MW of Blue 347, 4700 µg/ml (≈ 10 mM, the prescribed maximum concentration in most OECD guidelines for *in vitro* genotoxicity tests) in deionised water was applied as maximum concentration. The concentrations were further chosen based on toxicity data and on the occurrence of precipitation. In the absence of S9-mix V79 cells were treated for 24 h, in the presence of S9-mix for 4 h; cells were harvested 24 h after the beginning of treatment. The highest concentration should produce approximately 60% decrease in replication index. Negative and positive controls were included.

Results
Precipitation of Blue 347 in culture medium was observed at 2350 µg/ml and above in the absence of S9-mix and at 587.5 µg/ml and above in the presence of S9-mix. In addition no relevant increase in pH or osmolarity occurred. No clear cytotoxic effects indicated by a reduced proliferation index were found both in the absence and the presence of S9-mix.
In the absence of S9-mix biologically relevant and statistically significant increases in the number of V79 cells with micronuclei were found at the 3 highest concentrations tested. The increase did not show a clear concentration dependency. In the presence of S9-mix a biologically relevant and statistically significant increase in the number of V79 cells with micronuclei was only observed at a mid-concentration.

Conclusion
Under the experimental conditions used Blue 347 induced an increase in the number of V79 cells with micronuclei and, consequently, is genotoxic (clastogenic and/or aneugenic) in V79 cells.

Ref.: 8

Comment
Only a short report is available which only contains the "results and discussion" paragraph. Yet, the most relevant information is described. The study was not conducted in compliance with GLP or according to the draft OECD guideline. Purity is not mentioned. Although the same batch was reported to be 98.5% pure in other *in vitro* genotoxicty tests available. Although rat liver S9 fraction was used, the inducer chemical was not reported.
Despite these shortcomings, the SCCS considers the outcome of this study as relevant.

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

In vivo Mammalian Erythrocytes Micronucleus Test

Guideline:	OECD 474 (1997)
Species/strain:	mouse, NMRI
Group size:	5 males and 5 females per test group
Test substance:	Blue 347
Batch:	DYBB0657
Purity:	100% (HPLC)
Vehicle:	0.9% NaCl
Dose level:	0, 6.25, 12.5 and 25 mg/kg bw
Route:	i.p., once
Sacrifice times:	24 h and 48 h (high dose only) after treatment

GLP: in compliance
Study period: 27 September 2006 – 15 November 2006

Blue 347 has been investigated for induction of micronuclei in the polychromatic erythrocytes of mice. Test doses were based on the results of a preliminary study on acute toxicity performed under identical conditions as in the mutagenicity study. Male and female mice were treated i.p. with 10 up to 100 mg/kg bw and examined for acute toxic symptoms and/or mortality at 1, 2-4, 6, 24, 30 and 48 h after each treatment. In the main experiment female mice were exposed orally to 0, 6.25, 12.5 and 25 mg/kg bw. The mice were examined for acute toxic symptoms and/or mortality at 1, 2-4, 6 and 24 h after treatment. Bone marrow cells were collected 24 h or 48 h (highest dose only) after dosing. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatic (PCE/NCE). Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-test the mice treated i.p. with 100 mg/kg bw died within 1 h after treatment. After treatment with 50 mg/kg bw, 1 mouse died after 6 h. The surviving mice showed reduction in spontaneous activity, abdominal position and ruffled fur at least the first 6 h after treatment. At the lower doses these clinical effects were less obvious. Mice treated with doses of 25 mg/kg bw and above had blue coloured urine. In the micronucleus test, 2 the mice showed a reduction spontaneous activity and ruffled fur as well as coloured urine even down to the lowest dose of 6.25 mg/kg bw.
A decrease in the PCE/NCE ratio was not observed at both sampling times. However, the clinical signs reported, particularly the coloured urine, indicated systemic distribution and thus bioavailability of Blue 347.
A biologically relevant increase in the number of cells with micronuclei was not observed at any sampling time and dose level of Blue 347.

Conclusions
Under the experimental conditions used Blue 347 did not induce an increase in the number of bone marrow cells with micronuclei and, consequently, Blue 347 is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 9

3.3.7 Carcinogenicity

No data submitted

3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

No data submitted

3.3.8.2 Teratogenicity

Dose range-finding study

Guideline: /
Species/strain: rat, HanRcc:WIST(SPF)
Group size: 5 mated females per test group
Test substance: Blue 347
Batch: DYBB0847
Purity: 97% (HPLC)
Vehicle: Milli-Q-water

Dose levels:	0, 100, 300, 1000 mg/kg bw/d
Dose volume:	10 mL/kg bw
Route:	oral (gavage)
Administration:	day 6 – 20 *post-coitum*
GLP statement:	/
Study period:	22 January – 22 February 2008

The purpose of this study was to detect effects on the pregnant rat and development of the embryo and foetus consequent to exposure of the female to the test item from day 6 post coitum (implantation) to Day 20 post coitum (the day prior to Caesarean section). A standard dose volume of 10 mL/kg body weight with a daily adjustment to the actual body weight was used. Control animals were dosed with the vehicle alone (Milli-Q-Water).

Results
All females survived until the scheduled necropsy. Treatment with the test item up to and including 1000 mg/kg bw/d resulted in no clinical findings except blue discoloured faeces. At 300 and 1000 mg/kg bw/d, mean food consumption was reduced during the treatment period, resulting in reduced mean body weight gain, at 1000 mg/kg bw/d also the mean absolute body weights were reduced. Post-implantation losses and the mean number of foetuses per dam were not affected by treatment with the test item at any dose level. During necropsy of the females, no macroscopical findings were noted. During the external examination of the foetuses, no external findings were noted. No test item-related effects on foetal sex ratios were noted. No test item-related effects on foetal body weights were noted.

Conclusion
Based on these results, the NOAEL (No Observed Adverse Effect Level) for maternal and foetal organisms was considered to be 100 mg/kg bw/d. Based on these data the following dose levels are considered appropriate for the main study: 40, 200 and 1000 mg/kg bw/d.
Ref.: 12

Main study

Guideline:	OECD 414 (2001)
Species/strain:	rat, HanRcc:WIST(SPF)
Group size:	22 mated females per test group
Test substance:	Blue 347
Batch:	XB20178
Purity:	97%
Vehicle:	Milli-Q-water
Dose levels:	0, 40, 200, 1000 mg/kg bw/d
Dose volume:	10 mL/kg bw
Route:	oral (gavage)
Administration:	daily from day 6 – 20 *post-coitum*
GLP statement:	in compliance
Study period:	9 June – 8 October 2008

88 mated female rats, at least 11-week old at pairing, 22 per group, were used. The body weight of the animals in this test was between 190 - 237 grams. After homogenizing in bi-distilled water the analysis of homogeneity and stability of Blue 347 in bi-distilled water was performed before administration to the animals. The test item was also found to be stable in the vehicle for at least 7 days when stored at room temperature (20 ± 5 °C). The mixture of the test article and vehicle were prepared weekly. According to the result of dose range finding study the following doses were used for the study: 0, 40, 200, 1000 mg/kg bw/d. The animals (22 mated female rats, each concentration) were treated by oral gavage, once

daily from day 6 through to Day 20 post coitum. A standard dose volume of 10 ml/kg bw was used. Females were sacrificed on day 21 post coitum and the foetuses were removed after Caesarean section. The examination of the dams and foetuses was performed in accordance with international recommendations. Due to skeletal staining error, caused by a lack of homogeneity in the stain used for processing, 8 foetuses could not be evaluated for bone or cartilage parameters. A further 17 foetuses could not be reliably evaluated for "Bone ossification stage/supernumerary ribs". Additionally, in two of these foetuses, one finding recorded at examination for "bone and cartilage abnormalities and variations" was considered unreliable. To demonstrate that the staining error does not affect the reliability of the study two different ways to report the results were applied:

Data Set A included all foetuses that could be reliably evaluated for each particular examination type. In this summary, incomplete litters have been included where some foetuses in a litter could be evaluated and other foetuses in the same litter could not be evaluated. *Data Set B* included only those litters in which there were no foetuses affected by processing error were included. Litters in which some foetuses could be evaluated and other foetuses could not be evaluated have been excluded from these summaries. All skeletal examinations results summarized under "result" are based on these summaries. Comparison of the two data sets did not show a difference in the result and in each group, a minimum of 16 complete litters were fully evaluated.

Results
All females survived until the scheduled necropsy. No signs of discomfort or clinical symptoms from treatment with the test item were noted. At 1000 mg/kg bw/d blue discoloured faeces were observed in all females. These discolorations were considered to be a consequence of the colouring properties of the test item and not adverse. Two females had blue discoloured fur and/or a blue discoloured tail during six and seven days at the beginning of the treatment period, respectively, which was possibly due to direct contact with discoloured faeces or bedding material. At 1000 mg/kg bw/d the mean food consumption was statistically significantly reduced from days 6 to 12 post coitum (up to 10.4% compared to the control group). The overall reduction of mean food consumption during the treatment period was 5.2% compared to the control group. This reduction was considered to be test item-related. In the other groups mean food consumption was not affected by the treatment with the test item. At 1000 mg/kg bw/d mean body weight gain was slightly but not statistically significantly reduced from day 13 post coitum onwards (24.8% compared to 26.8% in the control group until day 20 post coitum). This finding was considered to be test item-related. Mean body weights were similar to the control group and not affected by treatment with the test item. Mean corrected body weight gain (corrected for the gravid uterus weight) was slightly but not statistically significantly reduced at 1000 mg/kg bw/d (+12.3% compared to +14.5% in the control group), which was considered to be not test item-related. In the other groups mean body weight, mean body weight gain and mean corrected body weight gain were similar to the control group and not affected by the treatment with the test item.

There was no test item-related effect on the relevant reproduction parameters in any dose group. The mean number of post-implantation loss was 1.0, 0.7, 0.8 and 0.5 and the mean number of foetuses per dam was 12.3, 12.1, 12.3 and 12.5 in order of ascending dose-levels. During macroscopic examination, no test item-related adverse effects were noted in any dose group. At 1000 mg/kg bw/d in all females the intestinal content was discoloured blue. In the groups 40 and 200 mg/kg bw/d, for 1 and 13 females a blue discoloured intestinal content was noted, respectively. These discolorations were considered to be a consequence of colouring properties of the test item and not of adverse character.
No findings were noted during external examination of the foetuses in any group. No test item-related effects on the sex ratio of the foetuses and mean foetal weights were noted in any group.
During visceral examination of the foetuses no test item-related findings were noted.

During skeletal examination of the foetuses, no test item-related findings were noted. The type and frequencies of the noted less common variations were similar in nature for the groups receiving the test item and the control group and did not indicate any dose-dependency, therefore they were considered not to be test item-related. There were no test item-related effects on the ossification stage and the number of supernumerary ribs in any dose group. When calculated on a foetus basis, several incompletely or non-ossified bones affecting the cranium, cervical vertebrae, sternum and hind limbs were noted in all dose groups. These occasionally statistically significant differences showed no dose dependency and were caused by advanced as well as retarded stages of development. Furthermore, these effects were not present on litter basis and were therefore considered not to be test item-related. The number of supernumerary ribs was similar in all groups. At 1000 mg/kg bw/d there was a statistically significantly higher incidence of rudimentary supernumerary left ribs, when calculated on a foetus basis. Since this higher incidence was in the range of the historical control data this finding was considered to be incidental.

Conclusion
At 1000 mg/kg bw/d, blue discoloured faeces were observed in all females, which were considered to be a consequence of the colouring properties of the test item. Mean food consumption was statistically significantly reduced from days 6 to 12 post coitum, resulting in a slightly reduced mean body weight gain from day 13 post coitum onwards. Treatment with the test item up to and including 1000 mg/kg bw/d did not affect embryo-foetal development.
Based on the above mentioned results, for maternal organisms the NOAEL (No Observed Adverse Effect Level) was considered to be 1000 mg/kg bw/d and the NOEL (No Observed Effect Level) was considered to be 200 mg/kg bw/d. The NOEL for foetal organisms was considered to be 1000 mg/kg bw/d. Under the conditions described for this study, Blue 347 did not reveal teratogenic potential up to and including 1000 mg/kg bw/d.

Ref.: 13

Comment
It is not clear why the study authors considered the reduction of both the mean food consumption and the mean body weight gain at 1000 mg/kg bw/d as test item related and, in contrast, the reduction of the corrected body weight gain as not being test item related. The SCCS sets the NOAEL of maternal toxicity at 200 mg/kg bw/d, the NOAEL of developmental toxicity including teratogenicity is 1000 mg/kg bw/d.

3.3.9 Toxicokinetics

No data submitted

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2 Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11 Human data

No data submitted

3.3.12 Special investigations

No data submitted

3.3.13 Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

HC Blue 17

Non oxidative conditions

Absorption through the skin	**A**	**=**	**4.72 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**2.74 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose	**SAS x A x 0.001/60**	**=**	**0.05 mg/kg bw/d**
No Observed Adverse Effect Level (subchronic toxicity study, oral, rat Maternal toxicity in developmental toxicity study)	**NOAEL**	**=**	**200 mg/kg bw/d**
50% bioavailable *		**=**	**100 mg/kg bw/d**
MOS		**=**	**2000**

* standard procedure according to the SCCS's Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation.

Oxidative conditions

Absorption through the skin	**A**	**=**	**4.23 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**2.45 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose	**SAS x A x 0.001/60**	**=**	**0.04 mg/kg bw/d**
No Observed Adverse Effect Level (subchronic toxicity study, oral, rat) (Maternal toxicity in developmental toxicity study)	**NOAEL**	**=**	**200 mg/kg bw/d**
50% bioavailable *		**=**	**100 mg/kg bw/d**
MOS		**=**	**2500**

* standard procedure according to the SCCS's Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation.

3.3.14 Discussion

Physico-chemical properties
HC Blue 17 is used as a hair colouring agent up to 2% on-head concentration of in non-oxidative as well as in oxidative hair dye formulations.
Characterisation of HC Blue 17 in two of the 4 batches was not performed. Purity of HC Blue 17 is not determined adequately (varying results, %peak area HPLC and no reference standard used) in any of the four batches, and no study was submitted to demonstrate the reported purity of HC Blue 17 in two batches DYBB0539 and DYBB0657**.** The impurities in two batches of HC Blue 17 reported in Summary Submission are qualitatively/quantitatively

different than those found by an earlier analysis. There was no study report to document the impurities, in all batches of HC Blue 17, described in the Summary Submission.
HC Blue 17 is a secondary amine, and thus is prone to nitrosation and formation of nitrosamines. It should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb.

Log Pow of HC Blue 17 has not been measured. Water solubility of HC Blue 17 has not been determined by EU method. No additional physico-chemical properties such as melting point, density, etc. have been reported. Stability of HC Blue 17 in typical hair dye formulation has not been documented

Irritation, sensitisation
Neat HC Blue 17 is not irritant to skin and slightly irritant to the eyes.
The highest concentration tested (15%) in the LLNA was considered too low. No conclusion regarding the sensitising potential of HC Blue 17 can be drawn.

Dermal absorption
The dermal absorption figure to be taken into consideration for the calculation of the margin of safety is the Mean + SD or 1.76 + 2.96 = 4,72 µg /cm^2 for non-oxidative conditions and (2.18 + 2.05 = 4.23 µg/cm^2) for oxidative condition, respectively.

General toxicity
No data on acute toxicity were submitted.
In the oral subchronic toxicity study in rats in the nasal cavity local irritant effects were observed which were concentration-related and also seen at the lowest dose 40 mg/kg bw/d. No NOEL was derived in this study. However, for the foreseen use as hair dye ingredient these effects are not considered relevant. Due to the intestinal lesions observed at 1000 mg/kg bw/d the NOAEL of systemic toxicity is 200 mg/kg bw/d.

The SCCS sets the NOAEL of maternal toxicity at 200 mg/kg bw/d, the NOAEL of developmental toxicity including teratogenicity is 1000 mg/kg bw/d. No study on reproductive toxicity was provided.

Mutagenicity
Overall, the genotoxicity of HC Blue 17 is sufficiently investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy.
HC Blue 17 did not induce gene mutations in a gene mutation test in bacteria nor in a gene mutation tests using the *hprt*-locus of mammalian cells. An *in vitro* gene mutation test using the *tk*-locus of mammalian cells was considered inconclusive. In an *in vitro* micronucleus test an increase in the number of V79 cells with micronuclei was observed.
The positive finding from the *in vitro* test was not confirmed in *in vivo* test. In an *in vivo* micronucleus test, HC Blue 17 exposure did not result in an increase in the number of bone marrow cells of mice with micronuclei.
Consequently, on the basis of these tests, HC Blue 17 can be considered to have no genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No data submitted

4. CONCLUSION

The SCCS is of the opinion that the use of HC Blue 17 at a maximum on-head concentration of 2.0% in both oxidative and non-oxidative hair dye formulations does not pose a risk to the health of the consumer.

The discrepancies and uncertainties related to the purity of the different batches should be clarified.

HC Blue 17 is a secondary amine, and thus is prone to nitrosation and formation of nitrosamines. It should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb.

A sensitising potential cannot be excluded.

5. MINORITY OPINION

Not applicable

6. REFERENCES

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