Scientific Committee on Consumer Safety

SCCS

OPINION ON

Basic Violet 2

COLIPA n° B115

The SCCS adopted this opinion at its 12th plenary meeting of 20 September 2011
About the Scientific Committees

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

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

SCCS

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

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm
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This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged.

Revised opinions carry the date of revision.
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1. BACKGROUND

Submission I for Basic Violet 2 with the chemical name 4-((4-Amino-3-methylphenyl)(4-imino-3-methyl-2,5-cyclohexadien-1-ylidene)methyl)-2-methylphenylamine monohydrochloride was submitted in September 2003 by COLIPA according to COLIPA.

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers adopted 23 April 2004 an opinion (SCCNFP/0784/04) with the conclusion that:

"The SCCNFP is of the opinion that the information submitted is inadequate to assess the safe use of the substance. Before any further consideration, the following information is required:

* complete physico-chemical characterisation of the test substances used;
* sub-chronic toxicity study providing a NOAEL;
* percutaneous absorption study in accordance with the SCCNFP Notes of Guidance;
* data on the genotoxicity/mutagenicity following the relevant SCCNFP-opinions and in accordance with the Notes of Guidance."

Basic Violet 2 is identical with CI 42520 also used as a colouring agent allowed for use in cosmetic products intended to come into contact only briefly with the skin.

Submission II of Basic Violet 2 was submitted by COLIPA in July 2005. According to this submission Basic Violet 2 is proposed to be used as a non-reactive hair colouring agents ("direct" dye) in oxidative and semi-permanent hair dye formulations at a maximum concentration of 0.25 and 0.5% in the finished cosmetic product, respectively.

An additional submission III was submitted in May 2008 applying for use of a higher concentration of this substance in oxidative hair dyes. The concentration applied for increase from 0.25% up to 1.0% on the scalp.

2. TERMS OF REFERENCE

1. Does SCCS consider Basic Violet 2 safe for use as an oxidative hair dye with a maximum concentration up to 1.0% on the scalp taken into account the scientific data provided?

2. Does SCCS consider Basic Violet 2 safe for use as a non-oxidative hair dye with a maximum concentration of 0.5% in the finished cosmetic product taken into account the scientific data provided?

3. And/or does the SCCS have any further scientific concerns with regard to the use of Basic Violet 2 in hair dye formulations?

1 COLIPA - European Cosmetics Toiletry and Perfumery Association
3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Basic Violet 2 (INCI name)

3.1.1.2. Chemical names

4-[(4-amino-m-tolyl)(4-imino-3-methylcyclohexa-2,5-dien-1-ylidene)methyl]-o-toluidine monohydrochloride
4-((4-Amino-3-methylphenyl)(4-imino-3-methyl-2,5-cyclohexadien-1-ylidene)methyl)-2-methylphenylamine-monohydrochloride
Benzenamine, 4,4'-(4-imino-3-methyl-2,5-cyclohexadien-1-ylidene)methylenebis[2-methyl-, monohydrochloride
Benzenamine, (4-amino-3-methylphenyl)(4-imino-3-methyl-2,5-cyclohexadien-1-ylidene)methyl]-2-methyl-, monohydrochloride

3.1.1.3 Trade names and abbreviations

Lowacryl Violet 2 (Lowenstein)
New Magenta
CI 42520
COLIPA B115

3.1.1.4 CAS / EC number

CAS: 3248-91-7
EC: 221-831-7

3.1.1.5 Structural formula

![Structural formula of Basic Violet 2]

3.1.1.6 Empirical formula

Formula: $C_{22}H_{23}N_3 \cdot HCl$

3.1.2 Physical form

Green powder
3.1.3 Molecular weight

Molecular weight: 365.91 g/mol

3.1.4 Purity, composition and substance codes

Chemical identity of batch 063-01/42-14 of the test substance with the code A 000945 has been tested using NMR and HPLC/MS/UV/VIS. 1H-NMR proved the identity of the test substance. The content of the test substance was 85.7% w/w. HPLC/ MS/ UV/VIS (254, 550nm) confirmed the identity of the test substance. Ref.: 1

Batch 063-01/41-06 had been tested in the same way as described above. The content of the test substance was 83.7%. Ref.: 2

For purity testing HPLC/MS/UV_VIS (DAD) has been used. Moreover water content (Karl Fischer); loss on drying (50°C; P2O5) and sulphated ash (NH4SO4; H2SO4) have been determined. Aniline, o- and p-toluidine were used for calibration and for the quantification of these possible impurities (254 nm). For the quantification of the homologues the sum of all HPLC peaks were set as 100% (550nm). Ref.: 1/2

3.1.5 Impurities / accompanying contaminants

Batch063-01/42-14

4-(Bis(4-amino-3-methylphenyl)methylene)-l-imino-2,5-cyclohexadiene-monohydrochloride (Carbol Fuchsin) 5.6% (550nm)
4-(Bis(4-aminophenyl)methylene)-l~imino»2-methyl-2,5-cyclohexadiene~monohydrochloride (Basic Violet 14) 0.1% (550nm)
l-(Bis(4'-aminophenyl)methylene)-4-imino-2,5~cyclohexadiene~monohydrochloride (Basic Fuchsin) < 100 ppm

o-Toluidine2 0.001% (average)
p-Toluidine <0.0015%
Aniline <0.001%
Sulphated ash 0.33%
Loss on drying 6.5%
Water content 7.0%
Methyl acetate(for purification) 2.4%

Batch 063-01/41-06

4-(Bis(4-amino-3-methylphenyl)methylene)-l-imino-2,5-cyclohexadiene-monohydrochloride (Carbol Fuchsin) 5.6% (550nm)

2 o-Toluidine is a carcinogen cat 1B. This low concentration would be of no concern in a hair dye formulation.
4-(Bis(4-aminophenyl)methylene)-l-imino-2-methyl-2,5-cyclohexadiene-monohydrochloride (Basic Violet 14) 0.2% (550nm) 
l-(Bis(4’-aminophenyl)methylene)-4-imino-2,5-
cyclohexadiene-monohydrochloride (Basic Fuchsin) < 100 ppm

\[
\begin{align*}
&\text{o-Toluidine} & 0.0039\% \text{ (average)} \\
&\text{p-Toluidine} & < 0.0015\% \\
&\text{Sulphated ash} & 0.33\% \\
&\text{Loss on drying} & 6.2\% \\
&\text{Water content} & 8.5\% \\
&\text{Methyl acetate (for purification)} & 1.5\% \\
\end{align*}
\]

Ref.: 2 (1999)

**Taken from summary submission III 2008**

Heavy metal content:
- Arsenic  <5ppm
- Antimony  <5ppm
- Lead  <20ppm
- Cadmium  <10ppm
- Mercury  <5ppm

Comment
The concentrations given for the homologues of Basic Violet 2 are relative ones, resulting from a comparison of peak areas in the HPLC chromatogram.

Both batches according to the applicants were purified by GST (according to later information by the applicant, the purified batches (063-01/41-06, 063-01/41-14) were prepared by crystallization with a solvent mixture) before testing physico-chemical properties.

In the submitted data there was no information, if the batches of B115 used for toxicity testing and for commercial products are purified in the same way before use. Corresponding questions of SCCS were answered by the applicants (see discussion).

**3.1.6 Solubility**

**Taken from summary submission II 2005:**

- Soluble in water  2.2 weight % (pH 5.8)
- in DMSO  5 weight %
- in Methanol  8 weight %
- in Propylene glycol  5.5 weight %

The solubility in water was determined according to EC guideline 92/69/EC A.6 (ref 5)

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

Log \( P_{ow} \): 0.03 at pH 8.0 (buffered solution; 21 °C)

Partition coefficient was determined according to EC directive 92/69/EC A.8

Ref.: 3

**3.1.8 Additional physicochemical specifications**
Melting point: 280 °C (decomposition)
Boiling point: / 
Rel. self ignition: > 400 °C
Vapour pressure: < 1.0 x 10^{-7} hPa (20 °C)
Density: 1.147 g/ml (20 °C)
Surface tension (water): 59.5 mN/m (20 °C)
Flammability: not highly flammable
pH: 4.51 (saturated aqueous solution at 20 °C)
Oxidising properties: not expected to be oxidising
Refractive index: /

3.1.9. Stability

A solution of B115 in water (2%) has been tested for stability using UV spectroscopy. Up to seven days at room temperature in the absence of light the solutions showed recoveries of 98.5% to 101.5%

Ref.: 2

In the receptor fluids of skin absorption experiments B115 decomposed under the influence of light. Within 7 day the concentration decreased by 53%. This, however, was considered in the calculation of absorption values.

Ref.: 23

The applicants report that B115 was stable for 6 months in a common market formulation of the dye stuff (0.5%) in a common packaging at 40 °C. The concentrations measured after 3 and 6 months were 0.54 and 0.44% respectively. HPLC/DAD was used for the determinations.

Basic Violet 2 was also tested with respect to its H2O2 stability (under acidic conditions). Using in a 1:1 mixture of the cream formulation and welloxon 6%, the recoveries were 100.0% at t=0; 100.7% at t=30 min; and 98.9% after t=60 min.

Ref.: 13

Answering a corresponding question of SCCS, the applicants stated, that the application of B115 under oxidative conditions takes place under neutral to acidic conditions (pH 6.8 – 2.6).

General Comments on Physico-chemical characterisation

- No reference substances were used for the quantification of homologues of Basic Violet 2. The concentrations given are only relative semiquantative ones.
- o-Toluidine, which is present as an impurity, is a human carcinogen which penetrates skin (skin notation DFG). According to the two batches of the test substance which have been used for testing the physico-chemical properties, the formulations applied to the scalp would contain only minimal o-toluidine concentrations of up to 0.000042%.
- The batches used for identity- and purity- testing have been purified before analytical quantification.
- According to the applicant, Basic Violet 2 is a non reactive dye.
3.2. Function and uses

Basic Violet 2 is a non-reactive hair colouring agent. It is used up to 0.5% in non-oxidative hair dye-formulation.

Basic Violet 2 is used up to an on-head concentration of 1.0% in oxidative hair dye formulations.

It is also used as a colorant (CI 42520) in cosmetic products (intended to come into contact only briefly with the skin (5 ppm maximum concentration in the finished product)\(^3\)).

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCNFP/0784/04

| Guideline: | OECD 401 (1987) - limit test |
| Species/strain: | Rat, Sprague Dawley Hsd: SD strain |
| Group size: | 5 males + 5 females |
| Batch: | GST 063-01/41-06 |
| Dose: | 2000 mg/kg bw–(limit test) |
| Vehicle: | 0.5% CMC aqueous solution |
| GLP: | in compliance |
| Study date: | 2002 |

Results
Mortalities: 3 males died within 5 days of dosing.
Clinical signs: Piloerection, breathing difficulties, staining of fur, urine and faeces.

Guideline: OECD 401 (1987)
Species/strain: Rat, Sprague Dawley Hsd: SD strain
Group size: 5 males + 5 females per group
Batch: GST 063-01/41-06
Dose: 500, 1000 and 2000 mg/kg bw
Vehicle: 0.5% CMC aqueous solution
GLP: in compliance

Results
Mortalities: At 2000 mg/kg bw, 1 male and 1 female died within 48 hours of dosing. No mortalities at lower dose levels.
Clinical signs at 2000 mg/kg bw: piloerection, reduced activity, hunched posture, breathing difficulties, staining of fur, urine and faeces, swollen abdomen.

Conclusion
The oral LD50 in rats exceeded 2000 mg/kg bw.

Ref.: 15

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

**Taken from SCCNFP/0784/04**

Guideline: OECD 404 (1992)
Species/strain: New Zealand albino white rabbit
Group size: 3 females
Observ. Period: 72 hours
Test substance: Basic Violet 2, 500 mg, mixed with 1 ml of water
Batch: GST 063-01/41-06
Purity: 94%
Dose level: 0.5 g (4h contact under semi-occlusion)
GLP: in compliance
Study date: 2003

A gauze square with 0.5 g test material mixed to a paste with sterile water was placed on the shaved skin of three female rabbits and covered with a semi-occlusive dressing for 4 hours. After the 4-hour application time, the area was wiped with cotton wool soaked with water. The animals were checked daily for mortality and systemic symptoms. Skin reactions were evaluated 1, 24, 48, and 72 hours after removing the patches according to the Draize scoring system.

Results
No mortality and no systemic symptoms were recorded in any of the test animals. Erythema could not be assessed due to intense staining of the skin. No oedema was noted in any of the test animals. Measurement of skin-fold thickness after 72 hours revealed no differences between treated skin and untreated (naive) skin. Erythema assessments after a longer period (when discolouration of the skin had disappeared) have not been performed.

Conclusion
The mean irritation scores 24 to 72 hours after application were below the thresholds defined in Commission Directive 2001/59/EC for classification of the test compound as irritating to the skin.

Ref.: 17

3.3.2.2. Mucous membrane irritation

**Taken from SCCNFP/0784/04**

Undiluted test compound

Guideline: OECD 405 (1987)
Species/strain: New Zealand albino white rabbit
Group size: 1 female
Observ. Period: 24 hours
Test substance: Basic Violet 2
A 100 mg aliquot of the test substance was placed into the conjunctival sac of the right eye of the animal. Lids were then held together for a few seconds. The untreated left eye served as control. Ocular reactions were evaluated approximately 1 and 24 hours after instillation of the test article.

Results
Intense staining of the eye and adnexa occurred. Chemosis score 2 of the conjunctiva was reported 1 hour after dosing, increasing to score 4 at 24h. Discolouration of the nictitating membrane, possibly indicating corrosive actions of the test substance, was also noted at the 24h observation time. Marked to severe corneal opacity was apparent after 24 hours. The study was terminated after 24h. No indication of a systemic effect could be detected.

Conclusion
Based on these results, the undiluted test compound was irritant to eyes.

Ref.: 18

Diluted test compound

Guideline: OECD 405 (1987)
Species/strain: New Zealand albino white rabbit
Group size: 3 females
Observ. Period: 14 days
Test substance: Basic Violet 2, 1% aqueous solution
Batch: GST 063-01/41-06
Purity: 94%
Dose level: 100 mg
GLP: in compliance
Study date: 2002

0.1 ml of a 1% solution of the test article in distilled water was placed into the conjunctival sac of the right eye of each animal. Lids were then held together for a few seconds. The untreated left eye served as control. The animals were checked daily for mortality and signs of systemic toxicity. Ocular reactions were evaluated 1, 24, 48, and 72 hours after instillation of the test article. Further examinations were performed 7 and 14 days after instillation.

Results
There were no mortality and no clinical signs. Slight conjunctival irritation (discharge and chemosis) was seen in all three animals (all scores = 1.0), while conjunctival redness could not be evaluated 1 h post treatment due to a diffuse colour produced by the test substance. Conjunctival irritation was still present on day 7, but the animals were completely recovered on day 14. Iris and cornea were not affected.

Conclusion
Since the mean irritation scores 24 to 72 hours after application were below the thresholds defined in Commission Directive 2001/59/EC, a 1% solution of the test compound is considered not to be irritating to the eye.

Ref.: 19

3.3.3. Skin sensitisation
Taken from SCCNFP/0784/04

Magnusson & Kligman Maximisation test

Guideline: OECD 406 (1992)
Species/strain: Dunkin-Hartley guinea pig
Group size: 20 females in test group, 10 females in control group
Observ. Period: 25 days
Test substance: Basic Violet 2
Batch: GST 063-01/41-06
Purity: 94%
Dose levels: intradermal injection: 1% solution, dermal induction: 50% solution, dermal challenge: 50% solution (preliminary screening study available)
GLP: in compliance
Study date: 2003

The test group consisted of 20 female Guinea pigs, two control groups of ten female Guinea pigs each. In the first week of induction, the test group was treated with single intradermal injections of complete Freund's adjuvant/water mixture 1:1 (v/v), 1% of the test substance in sterile water and with 1% of the test substance emulsified in Freund's complete adjuvant. The negative control groups were treated with the adjuvant and the vehicle (sterile water) in the same manner. Seven days after injection, a 50% solution of the test substance in sterile water was dermally applied under occlusive dressing for 48 h to the area of the intradermal injections. The negative control group was treated with the vehicle alone. After a period of 2 weeks without treatment, sensitisation reactions were challenged in the test group as well as in one negative control group by dermal administration of the test substance in sterile water (50%, on one flank and vehicle alone on the contralateral flank) under occlusive dressing for 24 hours. 24 and 48 hours after removal of the patches the skin reactions were scored. Following the 48 hour examination at challenge, skin fold thickness of the treated sites was measured using a digital micrometer. Body weights were recorded on days 1 and 25 (termination of the study). Body weights were not affected by the test compound.

Results
As marked coloration prevented formal assessment of erythema at any time point during the main study, assessment of skin fold thickness was performed. With the exception of one animal, values of the vehicle treated sites and test substance treated sites were in the same range in animals of the test group compared to animals of the control group.

Ref.: 20

Comment
The test is inconclusive due to staining of the skin.

Buehler test

Species/strain: Dunkin-Hartley guinea pig
Group size: 20 females in test group, 10 females in control groups
Observ. Period: 25 days
Test substance: Basic Violet 2
Batch: GST 063-01/41-06
The test group consisted of 20 female Guinea pigs, two control groups of ten female Guinea pigs each. During the induction phase, the test group was treated with the test substance in sterile water at 75% at the left flank. The negative control groups were treated with the vehicle (sterile water) in the same manner. The gauze patches with test substance or vehicle under occlusive dressing were removed after 6 hours. Approximately 24 hours after removal of the patches, skin reactions were scored. These procedures were repeated at weekly intervals (days 8/9 and 15/16 of the study).

On study day 29, sensitisation reactions were challenged in the test as well as in one negative control group by topical administration of the test substance in sterile water (50% on one side and vehicle alone on the contralateral flank) under occlusive dressing for 6 hours. Twenty-four and 48 hours after removal of the patches the skin reactions were scored. Following the 48 hour examination at challenge, skin fold thickness of the treated sites was measured using a digital micrometer.

Body weights were recorded on days 1 and 31 (termination of the study). Body weights were not affected by the test compound.

Results

As intense staining of the skin prevented formal assessment of erythema at any time point during the main study, assessment of skin fold thickness was performed. No differences between values of the test group compared to values of the control group were apparent. Slightly thicker skin at vehicle treated sites compared to test substance treated sites in both treated and control animals was regarded to be due to the different location (vehicle sites being anterior to test substance site).

Two reliability checks with mercaptobenzothiazole are stated at the end of the test: one generated 30% response in the test group and 0% response in the control group and the second check revealed 70% response in the test group and 70% response in the control group. Both were found acceptable by the performing laboratory.

Conclusion

The performing laboratory concludes that the test compound is not sensitising.

Comment

Due to intense staining of the skin, both experiments are inconclusive.

### 3.3.4. Dermal / percutaneous absorption

#### Non-oxidative conditions

<table>
<thead>
<tr>
<th>Guideline:</th>
<th>OECD 428</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue:</td>
<td>pig skin</td>
</tr>
<tr>
<td>Group size:</td>
<td>1 donor, 5 chambers</td>
</tr>
<tr>
<td>Skin integrity:</td>
<td>penetration tritiated water</td>
</tr>
<tr>
<td>Diffusion cell:</td>
<td>flow through Teflon-chambers</td>
</tr>
<tr>
<td>Test substance:</td>
<td>Basic violet 2</td>
</tr>
<tr>
<td>Batch:</td>
<td>GST063-01/41-06</td>
</tr>
<tr>
<td>Purity:</td>
<td>HPLC: 94.2 area% (m/z 330) at 550 nm</td>
</tr>
<tr>
<td></td>
<td>HPLC: 5.6 area% (m/z 316) at 550 nm</td>
</tr>
<tr>
<td></td>
<td>HPLC: 0.2 area% (m/z 302) at 550 nm</td>
</tr>
</tbody>
</table>
Radiochemical: tritiated water  
Test item: Color cream formulation for direct hair dyeing (0.5% Basic violet 2)  
Dose volume: 100 mg/cm²  
Receptor fluid: phosphate buffer  
Solubility receptor fluid: 0.116 mg/ml (pH 7.3)  
Stability receptor fluid: significant decomposition of 53% after 7 days  
Method of Analysis: HPLC  
GLP: in compliance  
Study date: 30 May – 6 June 2005

The cutaneous absorption of Basic Violet 2 in a typical hair dye formulation for direct hair dyeing was investigated in vitro using pig skin preparation. 400 mg of a hair dye formulation containing 0.5% Basic Violet 2 were applied to 4 cm² pig skin. The formulation was removed after 60 minutes.

Results
All receptor fluid samples were below the limit of detection (1.5 ng per injection). The applicant considered for the worst case assumption a maximal amount of 0.01 ± 0.00 µg/cm² of Basic Violet 2 as biologically available.

Ref.: 23

Comment
The amount applied was very high and only 5 chambers from one donor were used. There was a significant decomposition of the test substance in the receptor fluid which was corrected by the recovery rate.
The study is inadequate.

Submission 2011

Non-oxidative conditions

Guideline: OECD 428  
Tissue: human skin (abdomen or breast; thickness 380-400 µm)  
Group size: 10 chambers (from 5 donors)  
Skin integrity: penetration tritiated water  
Diffusion cell: Automated PTFE flow-through chambers (0.64 cm²)  
Test substance: Basic violet 2  
Batch: GST063-01/41-06  
Purity: HPLC: 93.8 area% at 250 nm  
Radiochemical: tritiated water  
Test item: Hair dye formulation under non-oxidative conditions (0.5% Basic violet 2)  
Dose volume: 20 mg/cm²  
Receptor fluid: phosphate buffered saline (containing sodium azide 0.01%, ethanol 5%)  
Solubility receptor fluid: 22.2 mg/ml in water  
Stability receptor fluid: /  
Method of Analysis: LC-MS/MS  
GLP: in compliance  
Study date: 22-25 October 2010

The percutaneous absorption of Basic Violet 2 in representative commercial hair dye formulation with an on-head concentration of 0.5% was performed under non-oxidative conditions using human skin in vitro. The receptor fluid was pumped through the receptor chamber at a rate of 1.5 ml/h. Thirty minutes after application, the test item was removed by
washing the skin with water. Receptor fluid was collected in 30 min fractions from 0 to 1 h post dose and hourly fractions from 1 to 6 h post dose and then in 2 hourly fractions from 6 to 72 h post dose.

Results

The dermal absorption of Basic violet 2 in non-oxidative conditions was 0.03 ± 0.01 µg/cm².

Ref.: 1 (subm 2011)

Comment
The mean + 1 SD (0.04 µg/cm²) should be used for the calculation of the Margin of Safety.

Oxidative conditions

Guideline: OECD 428
Tissue: porcine ear skin, 12 donors
Group size: 6/group (2 groups)
Skin integrity: conductivity
Diffusion cell: glass diffusion chamber
Test substance: Basic violet 2
Batch: FD019ST
Purity: 90% (HPLC, rel.)
Test item: Basic violet 2 (3%) in formulation
Dose volume: 20 µl/cm²
Receptor fluid: saline (0.9% NaCl)
Solubility receptor fluid: /
Stability receptor fluid: /
Method of Analysis: HPLC
Validation: limit of detection 2 ng/ml and limit of quantitation 2.5 ng/ml
GLP: in compliance
Study date: 23 – 27 April 2007
Two independent experiments were performed on fresh dermatomed pig skin samples under static conditions with 6 diffusion cells per experiment. Per diffusion cell, the skin of one animal was used. 20 µL of the mixed test item formulation were applied on each skin sample, left on the skin for 30 minutes and then washed off. The dermal absorption was monitored over 24 hours under non-occluded static conditions.

The test item was mixed with the colorant lotion at the ratio 1 to 2 prior to treatment. The mixture, corresponding to a final concentration of B115 of 1% was applied to the skin.

Results

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Chamber</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount of Basic Violet 2 (reference material) applied (µg)</td>
<td>153934</td>
<td>152861</td>
<td>154357</td>
<td>155348</td>
<td>147294</td>
<td>150425</td>
<td></td>
</tr>
<tr>
<td>Total amount of Basic Violet 2 (reference material), measured (µg)</td>
<td>162549</td>
<td>141044</td>
<td>127785</td>
<td>135411</td>
<td>130266</td>
<td>170886</td>
<td></td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>105.6</td>
<td>92.4</td>
<td>82.8</td>
<td>87.2</td>
<td>88.4</td>
<td>113.0</td>
<td></td>
</tr>
<tr>
<td>Dermal absorption of Basic Violet 2 (reference material), penetrated (ng/cm²)²</td>
<td>8.88</td>
<td>8.59</td>
<td>21.2</td>
<td>5.93</td>
<td>27.4</td>
<td>36.1</td>
<td></td>
</tr>
<tr>
<td>Dermal absorption (%)²</td>
<td>0.00377</td>
<td>0.00553</td>
<td>0.03137</td>
<td>0.00382</td>
<td>0.0186</td>
<td>0.0233</td>
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<th>Chamber</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>Amount of Basic Violet 2 (reference material) applied (µg)</td>
<td>-</td>
<td>179450</td>
<td>163131</td>
<td>167035</td>
<td>161367</td>
<td>164164</td>
<td>170972</td>
</tr>
<tr>
<td>Total amount of Basic Violet 2 (reference material), measured (µg)</td>
<td>169169</td>
<td>153707</td>
<td>158126</td>
<td>159290</td>
<td>175425</td>
<td>171834</td>
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<tr>
<td>Recovery (%)</td>
<td>94.0</td>
<td>100.4</td>
<td>94.7</td>
<td>98.1</td>
<td>108.8</td>
<td>100.0</td>
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<tr>
<td>Dermal absorption of Basic Violet 2 (reference material), penetrated (ng/cm²)²</td>
<td>8.80</td>
<td>14.2</td>
<td>11.9</td>
<td>26.3</td>
<td>19.9</td>
<td>342</td>
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<tr>
<td>Dermal absorption (%)²</td>
<td>0.00396</td>
<td>0.00866</td>
<td>0.00710</td>
<td>0.0163</td>
<td>0.0122</td>
<td>0.200</td>
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<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermal Absorption (ng/cm²)</td>
<td>8.88</td>
<td>8.59</td>
<td>21.2</td>
<td>5.93</td>
<td>27.4</td>
<td>35.1</td>
<td></td>
</tr>
<tr>
<td>Dermal Absorption (%)</td>
<td>0.00577</td>
<td>0.00563</td>
<td>0.0137</td>
<td>0.00382</td>
<td>0.0186</td>
<td>0.0233</td>
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<table>
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<tr>
<th>Experiment 2</th>
<th>Chamber</th>
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<th>3</th>
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</tr>
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<tbody>
<tr>
<td>Dermal Absorption (ng/cm²)</td>
<td>8.80</td>
<td>14.2</td>
<td>11.9</td>
<td>26.3</td>
<td>19.9</td>
<td>342</td>
<td></td>
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<tr>
<td>Dermal Absorption (%)</td>
<td>0.00399</td>
<td>0.00868</td>
<td>0.00710</td>
<td>0.0163</td>
<td>0.0122</td>
<td>0.200</td>
<td></td>
</tr>
<tr>
<td>Mean dermal absorption experiment I-II ± SD</td>
<td>0.0273 ± 0.0575 %</td>
<td>0.0461 ± 0.0986 µg/cm²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusion
The dermal absorption of Basic Violet 2 in oxidative conditions is $0.0461 \pm 0.0986 \, \mu g/cm^2$ or $0.0278 \pm 0.0575\%$.

Comment
The mean + 1SD ($0.145 \, \mu g/cm^2$ ($0.046 + 0.099$)) should be used for the calculation of the Margin of Safety.

The sponsor stated that the study was performed in oxidative conditions by adding 2 parts of a commercially available colour lotion, but the composition of the lotion was not reported.

### 3.3.5. Repeated dose toxicity

#### 3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

No data submitted

#### 3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

*Taken from SCCNFP/0784/04*

**Guideline:** OECD 408 (1998)

**Species/strain:** rat, Sprague Dawley Hsd: SD strain

**Group size:** 10 males + 10 females per group

**Batch:** GST 063-01/41-06

**Purity:** 94%

**Dose levels:** 3, 10 and 30 mg/kg bw/day

**Vehicle:** Water (10 ml/kg bw/day)

**Exposure:** once daily for 13 weeks

**Route:** oral, gavage

**GLP:** in compliance

**Study date:** 2002

**Results**

**Mortality:** Two high-dose females died on day 57, probably due to misdosing.

**Clinical signs:** Staining of the faeces in all test groups. Occasional hunched posture and dyspnoea in a few high-dose rats

**Neurotoxicity:** Motility impairment in a few high-dose rats

**Ophthalmoscopy:** No treatment-related findings

**Body weight:** Reduced body weight in high-dose females from day 8 of treatment

**Food intake:** Reduced food intake in high-dose females in the last week

Haematology, clinical chemistry and organ weights:
Macroscopy: Dark and firm areas in the liver in three high-dose males

Histopathology: Centrilobular hepatocytic hypertrophy in 5 mid-dose males and in most high-dose males and females. Hepatocytic necrosis in one high-dose male. Nephropathy (tubular basophilia/dilatation and/or chronic inflammation) in 5 mid-dose males and 6 high-dose males.

Conclusion
The changes in haematology and clinical chemistry in the low-dose group were stated to be ‘within the range of historical control data’, and a NOAEL of 3 mg/kg bw/day was concluded by the authors. However, taking into account the effects observed at higher dose levels and the dose-related responses, the SCCNFP concluded in 2004 that the NOAEL is below 3 mg/kg bw/day (SCCNFP/0784/04).

Ref.: 16

In the updated submission II of 2005, the 90-d study was re-evaluated by the applicant.
- With regard to body weight a statistically significant reduction was observed in females at 30 mg/kg bw and at 10 mg/kg bw/d also a reduction was noted. In males only a slight reduction was observed at 30 mg/kg bw. This was accompanied by reduced body weight gains in all these groups. In females at 30 mg/kg bw also food consumption was reduced.
- Terminal body weights were reduced in females at 30 mg/kg bw (statistically significant difference to controls). Relative organ weights were statistically significantly increased in males and females at 30 m/kg bw/d for liver, kidneys and heart, the latter being additionally elevated (statistically significant) in males at 10 mg/kg bw/d. In addition, at 30 mg/kg bw slight increases were observed in relative testes weights in males and relative brain weights in females (both statistically significant). Despite the results of the statistical analyses of differences to controls, the differences were slight and all mean values remained well within the range of historical control data. The only organ in which a histopathological correlate was observed was the liver (centrilobular hepatocytic hypertrophy in 5 mid-dose males and in most high-dose males and females. Hepatocytic necrosis in one high-dose male).
- Haematological observations were reduction in red blood cells counts in both males and females which were statistically significant at 10 and 30 mg/kg bw/d for males and at 30 mg/kg bw/d for females. Furthermore, haematocrit values were slightly reduced in all
dose groups in females. Further, the values for mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) were slightly increased in males and females of all dose groups. However, all changes were in the range of the historical controls of the test institute.

- In the clinical biochemical investigations some differences were observed with regard to Na, Ca, K, glucose, cholesterol as well as in the levels of alkaline phosphatase and aspartate aminotransferase. It was argued that all values were well within the range of historical controls.

Comment of the SCCS on sub-chronic toxicity
At the doses 30 and/or 10 mg/kg bw/d histopathological findings in liver and kidney indicate organ toxicity which is supported by changes in body and organ weights as well as in biochemical parameters (cholesterol, aspartate aminotransferase, alkaline phosphatase). The NOAEL is considered to be 3 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**

| Species/Strain: | *Salmonella typhimurium*: TA98, TA100, TA102, TA1535, TA1537 |
| Replicates: | triplicates in two independent experiments |
| Test substance: | Basic Violet 2 |
| Batch: | GST063-01/41-06 |
| Purity: | HPLC: 94.2%; NMR: 96.8%; |
| Vehicle: | DMSO |
| Concentration: | experiment 1: 1, 10, 100, 300, 1000 and 5000 µg/plate with and without S9-mix  
experiment 2: 3, 10, 30, 100, 300, 1000 and 3000 µg/plate with and without S9-mix |
| Treatment: | direct plate incorporation with 48 h incubation without and with S9-mix |
| GLP: | in compliance |
| Study period: | 14 July 2000 – 2 August 2000 |

Basic Violet 2 was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Both experiments were performed with the direct plate incorporation method. Negative and positive controls were in accordance with the OECD guideline.

**Results**

Toxicity observed as growth inhibition, clearing of the background lawn and strong reduction in the number of spontaneous revertants, was found in the first experiment in the absence of S9-mix for TA98 and TA102 (1000 and 5000 µg/plate), TA100, TA1535 and TA1537 (100, 1000 and 5000 µg/plate); in the presence of S9-mix for TA98 (5000 µg/plate), TA100 and TA1535 (100, 1000 and 5000 µg/plate) and TA1537 and TA102 (1000 and 5000 µg/plate). This toxicity was observed also in the second experiment. An increase in the number of revertants was observed on TA102 strain only in the presence of S9-mix at non-toxic concentrations. However, the effect never reached a factor of 2 compared to the control. This effect was not considered biologically relevant. The study
must be considered inadequate for the evaluation of this substance, because of the substantial toxicity observed which made an adequate evaluation of the mutagenic effect of this substance on *Salmonella typhimurium* impossible.

**Conclusion**

Under the experimental conditions used Basic Violet 2 was not mutagenic in this gene mutation tests in bacteria.

Ref.: 26

**In Vitro Mammalian Cell Gene Mutation Test**

<table>
<thead>
<tr>
<th>Guideline:</th>
<th>OECD 476 (1997)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species/strain:</td>
<td>mouse lymphoma cell line L5178Y tk&lt;sup&gt;+&lt;/sup&gt;/-</td>
</tr>
<tr>
<td>Replicates:</td>
<td>duplicate cultures in 2 independent experiments</td>
</tr>
<tr>
<td>Test substance:</td>
<td>Basic Violet 2</td>
</tr>
<tr>
<td>Batch:</td>
<td>GST 063-01/41-06</td>
</tr>
<tr>
<td>Purity:</td>
<td>94 % (HPLC)</td>
</tr>
<tr>
<td>Vehicle:</td>
<td>DMSO</td>
</tr>
</tbody>
</table>
| Concentrations:    | experiment 1: 0.0625, 0.125, 0.25, 0.5, 0.75, 1 and 1.5 µg/ml without S9-mix 0.156, 0.313, 0.625, 1.25, 2.5 and 2.75 with S9-mix  
|                    | experiment 2: 0.025, 0.05, 0.1, 0.2, 0.3 and 0.4 µg/ml without S9-mix 1.5, 2, 2.5, 2.6 and 2.7 µg/ml with S9-mix |
| Treatment:         | experiment 1: 3 h both without and with S9-mix; expression period 7-9 days, selection growth 13-14 days  
|                    | experiment 2: 24 h without S9-mix; expression period 7-9 days, selection growth 13-14 days 3 h with S9-mix; expression period 7-9 days, selection growth 13-14 days |
| GLP:               | in compliance |
| Study period:      | 29 October 1999 – 4 July 2003 |

Basic Violet 2 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. Basic Violet 2 test concentrations were based on the results of a cytotoxicity assay with a wide range of concentration levels both in the presence and absence of S9-mix measuring cell survival relative to the concurrent vehicle control cell cultures. In the main test cells were treated for 3 h or 24 h (experiment 2 with S9-mix only) followed by an expression period of 7-9 days to fix the DNA damage into a stable *tk* mutation.

Toxicity was measured as percentage relative total growth of the treated cultures relative to the survival of the solvent control cultures. Negative and positive controls were in accordance with the OECD guideline.

**Results**

In the cytotoxicity test both in the absence and presence of S9-mix and at 3 and 24 h treatment, severe toxicity was observed at the highest dose levels tested, reducing survival below 5% of the negative control level. On the basis of these findings 1.5 and 2.75 µg/ml were selected as maximum dose level used in the first experiment with and without S9-mix respectively and 0.4 µg/ml in experiment 2 for the 24 h treatment group. In the 2 experiments in the absence of S9-mix the appropriate level of toxicity (reduction of the relative total growth after the highest dose to 10-20%) was reached; in the experiments
in the presence of S9-mix the appropriate level of reduction in relative total growth was not reached.
No biologically relevant and dose dependent increases in the mutant frequency following treatment with Basic Violet 2 were found at any dose level, in the absence or presence of S9-mix at any treatment time.

Conclusion Basic Violet 2
Under the experimental conditions used, Basic Violet 2 was not mutagenic in the mouse lymphoma assay at the tk locus.

Ref.: 27

3.3.6.2 Mutagenicity/Genotoxicity in vivo

**In vivo Mammalian Erythrocytes Micronucleus Test**

<table>
<thead>
<tr>
<th>Guideline:</th>
<th>OECD 474 (1997)</th>
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</thead>
<tbody>
<tr>
<td>Species/Strain:</td>
<td>Swiss CD-1 Mice</td>
</tr>
<tr>
<td>Group size:</td>
<td>5 mice/sex/dose</td>
</tr>
<tr>
<td>Test substance:</td>
<td>Basic Violet 2</td>
</tr>
<tr>
<td>Batch:</td>
<td>GST 063-01/41-06</td>
</tr>
<tr>
<td>Purity:</td>
<td>HPLC: 94% (HPLC)</td>
</tr>
<tr>
<td>Vehicle:</td>
<td>sterile distilled water</td>
</tr>
<tr>
<td>Route:</td>
<td>Intraperitoneal injection</td>
</tr>
<tr>
<td>Dose level:</td>
<td>0, 3, 6 and 12 mg/kg bw</td>
</tr>
<tr>
<td>Sampling:</td>
<td>24 and 48h control and high dose group only</td>
</tr>
<tr>
<td>GLP:</td>
<td>in compliance</td>
</tr>
<tr>
<td>Study date:</td>
<td>3 December 1999 – 19 June 2003</td>
</tr>
</tbody>
</table>

Basic Violet 2 was evaluated for its potential to induce micronuclei in bone marrow after ip administration of the test article to mice. Test doses were based on acute toxicity in a pre-test with 2 animals per sex/group. In the micronucleus test groups of 5 male and 5 female mice were treated with test material dissolved in sterile distilled water. The test article was administered intraperitoneally at 3, 6 and 12 mg/kg bw. Animals were inspected for signs of reaction to treatment daily throughout the study. Bone marrow cells were collected 24 h or 48 h (control and highest dose only) after dosing and stained with May-Gruenwald and Giemsa. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/PCE+NCE) over the negative control value. Negative and positive controls were in accordance with the OECD guideline.

Results
Three toxicity studies with decreasing doses were performed in order to identify the doses to be used in the main study. All mice died at doses ≥ 125 mg/kg bw. All mice treated with lower doses survived. Clinical signs observed were swollen abdomen, ataxia, hunched posture, lethargy, reduced activity, semi closed eyes and piloerection. Mice treated with 3.91 mg/kg bw did not show any adverse reactions. At doses ≥ 15.6 mg/kg bw the PCE/PCE+NCE ratio was markedly depressed. Therefore 12 mg/kg bw was chosen as top dose.

In the micronucleus test no animals died. Animals from the intermediate treatment group showed piloerection; those from the high dose group hunched posture, swollen abdomen, closed eyes, piloerection and pink spots in the cage litter indicative for excretion of coloured urine.

In the micronucleus test a reduction in the PCE/(PCE+NCE) ratio was observed for both sexes and both time points. Biological relevant increases in the number of bone marrow cells with micronuclei compared to the concurrent vehicle controls were not found at any dose tested, either 24 or 48 h after treatment or for males and females.

Conclusion
Under the experimental conditions, Basic Violet 2 did not induce an increase in bone marrow cells with micronuclei and, consequently, Basic Violet 2 is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 28

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

*Taken from SCCNFP/0784/04*

Guideline: OECD 414 (1983)
Species/strain: rat, Sprague Dawley Hsd: SD strain
Group size: 24 females (mated) per group
Batch: GST 063-01/41-06
Dose levels: 2, 10 and 50 mg/kg bw/day
Vehicle: distilled water (10 ml/kg bw/day)
Treatment period: days 6-15 of gestation
GLP: in compliance
Study date: 2001

Results
Clinical signs: Violet coloured faeces in mid- and high-dose groups. Dyspnoea in high-dose group
Body weights: reduced in high-dose group from day 8
Food intake: reduced in high-dose group during treatment
Necropsy F0: final body weight, uterus weight and corrected body weight were increased in high-dose group.
Litter data: The number of implantations was decreased in high-dose group. Foetal weight was decreased in the high-dose group.
Foetal visceral exam.: no treatment related effects
Foetal skeletal exam.: no treatment related effects

Conclusion
Foetal visceral and skeletal examination did not reveal teratogenic effects. Maternal toxicity and a delay of foetal development were observed at 50 mg/kg bw/day. The NOAEL for maternal and developmental effects was 10 mg/kg bw/day.

Ref.: 22

3.3.9. Toxicokinetics

*In vitro study with human intestinal epithelial cells*

Guideline: /
Cells: Human intestinal epithelial cell line TC-7
Test substance: Basic Violet 2
Batch no: GST063-01/41-06
Purity: 93.8 area % (HPLC, 254 nm)
The bioavailability of Basic Violet 2 across the intestinal barrier was investigated in human intestinal epithelial (TC-7) cells in vitro in 2 independent experiments. The permeability from the apical (A, pH 6.5) to the basolateral (B, pH 7.4) side was investigated at 37 °C in 96-well transwell plates with shaking for a 60 min contact time. Analysis of the donor (apical) and receiver (basolateral) samples was done by means of HLPC-MS/MS and the apparent permeability coefficient ($P_{app}$) was calculated. $^{14}$C-mannitol (about 4 µM) was used to demonstrate the integrity of the cell monolayer. Only monolayers revealing a permeability of $<2.5 \times 10^{-6}$ cm/sec were used. Propranolol, atenolol, vinblastine and ranitidine were analysed concurrently to demonstrate the validity of the test system.

According to the laboratory’s classification system, a low permeability is considered for test items revealing a $P_{app} < 2 \times 10^{-6}$ cm/sec. A $P_{app}$ of 2 - 20 $\times 10^{-6}$ cm/sec and a $P_{app} \geq 20 \times 10^{-6}$ cm/sec classify a substance to have a moderate and a high permeability, respectively. As recommended by FDA, ranitidine (50 % absorption in humans) was used as the low permeability reference compound and propranolol (90 % absorption in humans) was used as the high permeability reference compound.

Results
The total recovery for the reference substances and Basic Violet 2 ranged from 82 to 118 % (mean of 2 values). The permeability figures for the reference substances propranolol ($P_{app} = 50.6$ and $52.7 \times 10^{-6}$ cm/sec), a high permeability reference compound with 90 % absorption in humans, and ranitidine ($P_{app} = 0.3$ and $0.3 \times 10^{-6}$ cm/sec), known to be absorbed at about 50 % in humans, were within acceptance range of 20 - 60 $\times 10^{-6}$ cm/sec and 0 - 2 $\times 10^{-6}$ cm/sec, respectively and demonstrated the validity of the assay. For Basic Violet 2 a $P_{app}$ of $2.8 \times 10^{-6}$ cm/sec (2.9 and $2.6 \times 10^{-6}$ cm/sec) was determined and thus the test substance was classified to be of medium permeability, indicating a substantial absorption from the gastrointestinal tract.

Conclusion
A mean permeability in human intestinal epithelial (TC-7) cells of $2.8 \times 10^{-6}$ cm/sec was obtained with Basic Violet 2 which classifies the test item to be of medium permeability. As the absorption across the intestinal epithelium is considered to be the limiting factor of the uptake through the gastro-intestinal tract, the medium permeability observed in this assay indicates a substantial but not complete absorption of Basic Violet 2 after oral administration.

Comment
The study was not performed under GLP conditions, but a statement of the quality assurance unit of the test facility is included. There is no official guideline for this assay. However, the study was performed according to ECVAM recommendations. The generated data is considered to provide an estimation of the bioavailability of Basic Violet 2 after oral administration.

The substance is expected to be only partially absorbed after oral dosing, which should be considered in the calculation of the Margin of Safety.

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**

**Basic Violet 2**

*(non-oxidative conditions)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption through the skin A (µg/cm²)</td>
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</tr>
<tr>
<td>Skin Area surface SAS (cm²)</td>
<td>580 cm²</td>
</tr>
<tr>
<td>Dermal absorption per treatment SAS x A 0.001</td>
<td>0.023 mg</td>
</tr>
<tr>
<td>Typical body weight of human</td>
<td>60 kg</td>
</tr>
<tr>
<td>Systemic exposure dose (SED) SAS x A 0.001/60</td>
<td>0.0004 mg/kg bw/d</td>
</tr>
<tr>
<td>NOAEL</td>
<td>1.5 mg/kg bw/d</td>
</tr>
</tbody>
</table>

*(sub-chronic toxicity, oral, rat, corrected for 50% oral absorption)*

**MOS**

\[
\frac{\text{NOAEL}}{\text{SED}} = 3750
\]

*(oxidative conditions)*

<table>
<thead>
<tr>
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<th>Value</th>
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<tr>
<td>Skin Area surface SAS (cm²)</td>
<td>580 cm²</td>
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<tr>
<td>Dermal absorption per treatment SAS x A 0.001</td>
<td>0.084 mg</td>
</tr>
<tr>
<td>Typical body weight of human</td>
<td>60 kg</td>
</tr>
<tr>
<td>Systemic exposure dose (SED) SAS x A 0.001/60</td>
<td>0.0014 mg/kg bw/d</td>
</tr>
<tr>
<td>NOAEL</td>
<td>1.5 mg/kg bw/d</td>
</tr>
</tbody>
</table>

*(sub-chronic toxicity, oral, rat, corrected for 50% oral absorption)*

**MOS**

\[
\frac{\text{NOAEL}}{\text{SED}} = 1071
\]

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

3.3.14. Discussion

Basic Violet 2 is allowed to be used as a colorant at a maximum concentration of 5 ppm in cosmetic products intended to come into contact only briefly with the skin (Annex IV, part 1, CI 42520).
Physico-chemical specification
Basic Violet 2 is used up to 0.5% in non-oxidative hair dye formulation and up to 1.0% on-head concentration in oxidative hair dye formulations.

No reference substances were used for the quantification of homologues of Basic Violet 2. Their concentrations are relative semiquantitative ones.

The batches used for identity- and purity testing have been purified before the analytical determinations. Therefore SCCS raised the question, if the batches, which have been used for toxicity testing, had been purified in the same way before use and if this also applies for commercial products. The applicants stated that the batches used for toxicity testing had been purified in the same way and that the currently used quality shows higher purity compared to the batches described in the dossier.

General toxicity
The oral LD$_{50}$ of Basic Violet 2 in rats exceeded 2000 mg/kg bw. In a subchronic toxicity study in rats at the doses 30 and/or 10 mg/kg bw/d histopathological findings in liver and kidney indicate organ toxicity which is supported by changes in body and organ weights as well as in biochemical parameters (cholesterol, aspartate aminotransferase, alkaline phosphatase). The NOAEL is considered 3 mg/kg bw/d. However, the substance is expected to be only partly absorbed after oral application. Therefore the NOAEL was corrected to 1.5 mg/kg bw/d in the calculation of the Margin of Safety.

In a developmental toxicity study in rats, foetal visceral and skeletal examination did not reveal teratogenic effects. Maternal toxicity and a delay of foetal development were observed at 50 mg/kg bw/day. The NOAEL for maternal and developmental effects was 10 mg/kg bw/day.

No study on reproductive toxicity was submitted.

Toxicokinetics
A mean permeability in human intestinal epithelial (TC-7) cells of 2.8 x 10$^{-6}$ cm/sec was obtained with Basic Violet 2 which classifies the test item to be of medium permeability. As the absorption across the intestinal epithelium is considered to be the limiting factor of the uptake through the gastro-intestinal tract, the medium permeability observed in this assay indicates a substantial but not complete absorption of Basic Violet 2 after oral administration.

Irritation, sensitisation
The assessment of skin irritation potential was hampered because erythema could not be assessed due to intense staining of the skin. No oedema was noted in any of the test animals. Measurement of skin-fold thickness after 72 hours revealed no differences between treated skin and untreated (naive) skin. The undiluted test substance was therefore not considered a skin irritant.

The ocular irritation test with the undiluted substance showed clear eye damage. A 1% solution of the test compound is considered to be not irritating to the eye.

The sensitisation tests are inconclusive. A sensitisation potential of Basic Violet 2 cannot be excluded.

As a general remark about the skin irritation, eye irritation and sensitisation tests, all performed by the same laboratory, it can be stated that the Quality Assurance statements have dates which do not correspond with the actual dates of the performed studies, indicating that the studies on Basic Violet 2 have never been audited. Moreover, the final signature on GLP-compliance and Quality Assurance statements are dated 3 to 4 years after performance of the tests. Nevertheless, the studies are accepted for evaluation.
Dermal absorption
Under oxidative conditions, the mean + 1SD (0.145 µg/cm²) should be used for the calculation of the Margin of Safety. For non-oxidative conditions, the mean + 1SD (0.04 µg/cm²) should be used for the calculation of the Margin of Safety.

Mutagenicity
Overall, the genotoxicity of Basic Violet 2 is investigated for the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Only gene mutations were tested in vitro. Basic Violet 2 treatment did not result in an increase in gene mutations both in bacteria and in mammalian cells. The induction of chromosome aberrations and aneuploidy was only tested in an in vivo assay. In an adequate in vivo micronucleus test in mice an increase in bone marrow cells with micronuclei was not observed. Based on the data available, Basic Violet 2 can be considered to have no in vivo genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No data submitted

4. CONCLUSION

Based on the data provided, the SCCS is of the opinion that the use of Basic Violet 2 with a maximum on-head concentration of 1.0% in oxidative and 0.5% in non-oxidative hair dye formulations does not pose a risk to the health of the consumer.

A sensitisation potential of Basic Violet 2 cannot be excluded.

5. MINORITY OPINION

Not applicable

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