EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

Disperse Violet 1

COLIPA n° C64

Scientific Committees
on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion at its 7th plenary meeting

of 22 June 2010

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 Evaluation Agency (EMEA), 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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This opinion has been subject to a commenting period of four weeks after its initial publication. In case comments were received during this time, they 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 Disperse Violet 1, with the chemical name 1,4-Diaminoanthraquinone, was submitted in October 1999 by COLIPA [1, 2].

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted on the 19th plenary meeting of 27 February 2002 its opinion on Disperse Violet 1 (containing Disperse Red 15 as an impurity):

"*The SCCNFP is of the opinion that the information submitted is insufficient to allow An adequate risk assessment to be carried out. Before any further consideration, an allergenicity and an in vitro/in vivo percutaneous absorption study should be performed in accordance with the SCCNFP Notes of Guidance as well as an in vivo genotoxicity/mutagenicity study according to OECD Guidelines.*"

According to submission II, submitted by COLIPA in July 2005, Disperse Violet 1 is used as a non-reactive hair colour in semi-permanent hair dye formulations at a maximum on-head concentration of 0.5%.

Submission II presents updated scientific data on the above mentioned substance in line with the second step of the strategy for the evaluation of hair dyes http://ec.europa.eu/enterprise/sectors/cosmetics/files/doc/hairdyestrategyinternet_en.pdf) within the framework of the Cosmetics Directive 76/768/EEC.

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Safety (SCCS) consider Disperse Violet 1 safe for use as non-oxidative hair dye with a maximum on-head concentration of 0.5 % taking into account the scientific data provided?*

2. *Does the SCCS recommend any restrictions with regard to the use of Disperse Violet 1 in hair dye formulations?*

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association
[2] According to the records of COLIPA

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Disperse Violet 1 (INCI name)

3.1.1.2. Chemical names

9,10-Anthracenedione, 1,4-diamino- (CA INDEX NAME, 9CI)
1,4-Diamino-9,10-anthraquinone (IUPAC)
1,4-Diaminoanthraquinone

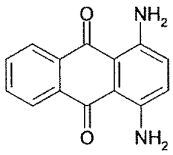
3.1.1.3. Trade names and abbreviations

Kayaku Violet 1
CI 61100
COLIPA n°: C064

3.1.1.4. CAS / EC number

CAS: 128-95-0
EC: 204-922-6

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{14}H_{10}N_2O_2$

3.1.2. Physical form

Dark violet powder

3.1.3. Molecular weight

Molecular weight: 238.25 g/mol

3.1.4. Purity, composition and substance codes

Characterisation of various batches of Disperse violet 1

Description of sample	MB002	305001 (R0015526)	BA14.05.90 (MN0390M)	WE 164/165 (HM 109/4)
Identity by H-NMR	confirmed	confirmed	confirmed	confirmed
Identity by IR	n/a	confirmed	confirmed	confirmed
NMR content / %, w/w	97.9	94.8	33.5[#]	27.5[#]
HPLC content / %, w/w	95.5	96.3[b]	27.5[a] (3.3% RSD)	26.4
HPLC purity (% peak area) 210 nm 254 nm 548 nm	 98.8* / 99.2*** 98.9* / 98.1*** 99.2* / 99.3***	 99.1** / 99.5*** 99.2** / 99.4*** 99.4** / 99.6***	 36.8 72.2 89.7	 38.6[c] 80.6[c] 96.5[c]
Content of 1-amino-4-hydroxy-anthraquinone [Disperse red 15] / %, w/w	0.88	0.93	3.0	1.2
Content of 1,4-bis-(methyl-amino)-anthraquinone [Disperse Blue 14] / ppm	n.d. LOD 10 mg/kg	n/a	n.d. LOD 8 mg/kg	n.d. LOD 10 mg/kg
Content of 1-methylamino-4-(2-hydroxyethyl)-amino-anthra-quinone [Disperse Blue 3] / ppm	n.d. LOD 30 mg/kg	n/a	n.d. LOD 23 mg/kg	n.d. LOD 30 mg/kg
Content of 1,4-dihydroxy-anthraquinone [Quinizarin] / %, w/w	0.05	0.07	0.24	0.11
Loss on drying / %, w/w	4.3	0.03	3.9	4.0
Water content / %, w/w	0.3	0.21	6.1	5.7
sulphated ash / %, w/w	0.47	0.58	18.9	17.8
starch	n/a	n/a	ca. 10	16.2
Na-Ligninsulfonate	n/a	n/a	60-70%[2]	67%[1]
Element screening	190 ppm Na, 41 ppm Mg, 150 ppm Si, 290 ppm Ca, 19 ppm Fe, 13 ppm, Zn, 800 ppm Br	266 ppm Na, 88 ppm Mg, 150 ppm Si, 450 ppm Ca, 35 ppm Fe, 19 ppm Zn, 0.21% Br	29 ppm B, 4.7% Na, 123 ppm Mg, 59 ppm Al, 635 ppm Si, 714 ppm K, 650 ppm Ca, 39 ppm Cr,13 ppm Mn, 846 ppm Fe, 17 ppm Ni, 0.176% Cu, 66 ppm Zn, 11 ppm Pb	23 ppm B, 5.57% Na, 152 ppm Mg, 69 ppm Al, 594 ppm Si, 329 ppm K, 0.127% Ca, 16 ppm Cr, 18 ppm Mn, 809 ppm Fe,10 ppm Ni, 0.119% Cu, 47 ppm Zn, 10 ppm Pb
Total mass balance / %	97.0	97.5	46	50

The NMR content should be just regarded as an indication due to signal interferences and solubility characteristics of the sample in dimethyl sulfoxide. Broad signals indicating proton containing organic compounds were detected both in the non-aromatic (3-4 ppm; 5 ppm) and aromatic (greater 6.8 ppm) range

[a] mean value from two independent requests

[b] corrected by mean HPLC content of reference substance (Ch. MB002, 95,5%)

[c] HPLC purity evaluated by isocratic elution

* from reference: A 2002/209

** from reference: A 2004/215

*** from reference: G09-A16145

[1] this is an approximate Na-Ligninsulfonate content. A non determined part is starch

[2] yield and identity was derived using a standard Na-ligninsulfonate

n/a not analysed

n.d not determined

Declarations by applicant

"Currently used quality" according to analytical report of the batch 305001 (R0015526), dated March 2009.

Purity
HPLC quantitative: > 95% w/w
NMR quantitative: > 94% w/w
Solvent content: < 5%
Ash: < 1%

Potential impurities
1-Amino-4-hydroxyanthraquinone (Disperse Red 15): < 1 % w/w
Quinizarin (1,4-Dihydroxyanthaquinone): ≤ 0.07% w/w
1,4-bis(methylamino)anthraquinone (Disperse Blue 14): < 10 ppm (LOD)
1-Methylamino-4-(2-hydroxyethyl)amino-anthraquinone (Disperse Blue 3): < 30 ppm (LOD)
Element screening: < 0.22% Br; < 20 ppm Zn; < 35 ppm Fe

Solvent residues
Solvents, (i.e. solvents such as methanol, ethanol, isopropanol, n-propanol, acetone, ethyl acetate, cyclohexane, methyl ethyl ketone and monochlorobenzene < 100 ppm) were not detected.

Batches used
MB002
305001 (R0015526)

"Former quality"

Purity
HPLC quantitative: > 26% w/w
NMR quantitative: > 26% w/w
Solvent content: < 8%
Ash: < 19%

Potential impurities
1-Amino-4-hydroxyanthraquinone (Disperse Red 15): < 3.1% w/w
Quinizarin (1,4-Dihydroxyanthaquinone): < 0.25% w/w
1,4- Bis(methylamino)anthraquinone (Disperse Blue 14): < 10 ppm (LOD)
1-Methylamino-4-(2-hydroxyethyl)amino-anthraquinone (Disperse Blue 3): < 30 ppm (LOD)
Starch: < 16.5% w/w
Element screening: < 30 ppm B; < 5.6% Na; < 160 ppm Mg; < 70 ppm Al; < 640 ppm Si; < 720 ppm K; < 0.13% Ca; < 40 ppm Cr; < 20 ppm Mn; < 850 ppm Fe; < 20 ppm Ni; < 0.180 % Cu; < 70 ppm Zn; < 11 ppm Pb

Solvent residues
Solvents, (i.e. solvents such as methanol, ethanol, isopropanol, n-propanol, acetone, ethyl acetate, cyclohexane, methyl ethyl ketone and monochlorobenzene < 100 ppm) were not detected.

Batches used
BA 14.05.90 (MN0390M)
HM 109/4 (WE164/165)

Note: Batch 960686 was not available for the comparative batch analysis.

"Deduced specification"

With the above mentioned information, the following specification for the raw material of Disperse Violet 1 was established:

HPLC quantitative	> 95% w/w
Disperse Red 15:	< 1% w/w
Quinizarin (1,4-Dihydroxyanthaquinone):	< 1000 ppm
Additional solvent content:	< 1%
Ash:	< 1%

Comments

Reported mass balance is not in agreement with the analytical results except for batch 305001 (R0015526)

Batch	Mass balance
MB002	97.9% NMR content + 0.88 Disperse red 15 + 4.3% loss on drying + others = >103%
	95.5% HPLC content + 0.88 Disperse red 15 + 4.3% loss on drying + others = >100%
BA14.05.90	27.5% HPL Content + 3.0% Disperse red 15 + 3.9% loss on drying + 10 % starch +
(MN0390M)	60% Na-ligninsulfonate + others = >104%
WE 164/165	26.4% HPLC content + 1.2% Disperse red 15 + 4 .0% water + 16.2 % starch +
(HM 109/4)	67% Na-ligninsulfonate + others = >114%

- Complete information on the composition of Disperse violet 1, Batch SO 1107787 used for the combined subchronic, teratogenicity, reproduction and dominant lethal assay from 1990 (Reference: 29), Batch 960686 used for acute toxicity and Batch CN 2121085 TSL no. 86-08 used for phototoxicity study (Reference 31) was not available. However, test batches of Disperse violet 1 used for the main studies for safety evaluation (skin sensitisation, dermal absorption, sub-chronic 90 days oral toxicity, and mutagenicity) are fully characterised.

- Based on the UV-absorption coefficient comparison, the applicant was able to demonstrate that the currently used Disperse Violet 1 has approximately 17% higher dye content than in the batches SO 1107787 and CN 2121085 TSL no. 86-08 used in 1980-1990. The test gives no information of the nature of impurities, but no different impurities would be expected because the synthesis route of Disperse Violet 1 was declared to be unchanged. The higher content and the high purity of the current quality infer that the currently used Disperse Violet 1 has less impurities than Disperse Violet 1 used in 1980-1990 for toxicological testing.

- As the NMR content was not reliable due to the poor solubility of C064, the HPLC content may be used for the adjustment of test-doses

3.1.5. Impurities / accompanying contaminants

See 3.1.4.

3.1.6. Solubility

Water:	0.16 mg/L, according to EC method A6
Ethanol:	0.1 weight %
DMSO:	9 % (w/w)
Acetone /water (1:1):	0.15%

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 2.55 (room temperature, pH 7.6), according to EC method A8

3.1.8. Additional physical and chemical specifications

Particle size distribution:	mean particle diameter: 73.0 µm (CIPAC MT59)
pH-value:	6.69 (saturated aqueous solution, 20°C)
Melting point:	266.9 – 267.4 °C
Boiling point:	not detectable, decomposition at 330 °C
Density:	1.443 g/ml (20 °C)
Vapour pressure:	4.7 exp -10 hPa (20 °C, extrapolated)
Surface tension (in water):	69.6 mN/m (20 °C)
Water solubility:	0.164 mg/l (20 °C)
Flammability:	not highly flammable
Explosive properties:	not expected to be explosive based on chemical structure
Relative self-ignition:	> 400 °C
Oxidising properties:	not expected to be oxidising based on chemical structure
UV-VIS Spectrum (200-800 nm):	/

3.1.9. Stability

The substance is considered to be stable for more than 5 years when stored dry and in the dark. The stability of Disperse Violet 1 in a 1:1 mixture of water/acetone (solution of 0.02% (w/v)) was monitored over a total time period of seven days using HPLC-chromatography at 548 nm detection wavelength. During the test procedure, all stock solutions were stored at ambient temperature in the absence of light. Disperse Violet 1 was found to be stable in that solvent under the conditions applied (Recovery at t=0: 100.0%; t=3h: 102.4%; t=2d: 97.8%; t =7d: 104.3%). The recovery rates are based on the zero time measuring point.

Ref.: 1 (subm. II)

The stability of Disperse Violet 1 in DMSO was tested over a period of 7 days. The test solutions (approximately 8% w/v) were stored at room temperature and in the absence of light. Disperse Violet 1 in DMSO was found to be stable (recovery at 0h = 100%, 6h = 98%, 2d = 99.7%, 7d = 98.6%).

General Comments to physico-chemical characterisation

- The data on purity and composition of several batches of Disperse Violet 1 test material was insufficient or incomplete, but the test batches used for the main studies for safety evaluation, in this opinion, were fully characterised
- The stability of Disperse Violet 1 in typical hair dye formulations is not provided.

3.2. Function and uses

Disperse Violet 1 is used as a hair colour in semi-permanent hair dye formulations at a maximum on-head concentration of 0.5%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCNFP/0504/01

Acute oral toxicity studies of 1,4-diamino-anthraquinone were performed in the rat and in the mouse. The calculated oral median lethal doses were:

- 3.50 g/kg bw for female rats, 3.30 g/kg bw for male rats and 820 mg/kg bw for mice.
  Ref.: 1 (subm. I)

- Above 5 g/kg bw in the rat.
  Ref.: 2 (subm. I)

- 1800 mg/kg bw in the rat.
  Ref.: 3 (subm. I)

- Between 1250 and 5000 mg/kg bw for males rats and between 625 and 1250 mg/kg bw for female rats.
  Ref.: 15 (subm. II)

Acute oral toxicity studies of 1-hydroxy–4-amino-anthraquinone were performed in the rat and in the mouse. The calculated oral median lethal doses were:

- 6.65 g/kg bw for female rats, 3.55 g/kg bw for female mice.
  Ref.: 34 (subm. I)

3.3.1.2. Acute intraperitoneal toxicity

Taken from SCCNFP/0504/01

An acute intraperitoneal toxicity study of 1,4-diamino-anthraquinone was conducted in NMRI mice. Median lethal dose for 1,4-diamino-anthraquinone was 640 mg/kg bw.
Ref.: 3 (subm. I)

3.3.1.3. Acute inhalation toxicity

Taken from SCCNFP/0504/01

An acute inhalation toxicity study of 1,4-diamino-anthraquinone was conducted in the rat**.** No mortality occurred, no adverse effects were recorded.
Ref.: 3 (subm. I)

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Taken from SCCNFP/0504/01

Guideline:	OECD 404 (1981)
Species/strain:	Pirbright albino guinea pigs
Group size:	5 female
Test substance:	Disperse violet 1
Batch:	Not given (purity not stated in study report)
Dose:	1 ml of a 1% aqueous formulation under closed patch
GLP:	not in compliance

The substance was suspended in water at a concentration of 1% and 1 ml was applied to 3 x 2 cm² area of intact skin of 5 female albino guinea pigs. Occlusive patches were applied and left in place for 4 hours. Reactions were evaluated after 24, 48, 72 and 144 hours.

Results
No cutaneous reactions were reported and the substance was classified as non-irritating to guinea pig skin at a concentration of 1% in water.

Ref.: 16 (subm. II)

In another study, 6 New Zealand white rabbits were used, and 500 mg Disperse Violet 1 was applied once as aqueous slurry to an area of 1 inch² of shaved intact skin. The material was left without occlusion for 24 hours, and the test reaction scored according to the Draize scale at 24 and 72 hours. All animals showed slight evidence of oedema and erythema at 24 hours, and none at 72 hours. The material was classified as a mild irritant (PII = 1.25) under these test conditions.

Ref.: 17 (subm. II)

3.3.2.2. Mucous membrane irritation

Taken from SCCNFP/0504/01

Guideline:	Not given
Species/strain:	New Zealand white rabbits
Group size:	4 female
Test substance:	Disperse Violet 1(100 mg) neat substance
Batch:	Not given (purity not stated in study report)
Dose:	100 mg neat substance
GLP:	not in compliance

The test substance was instilled in the conjunctival sac of the left eyes of the rabbits, and the right eyes were untreated. The eyes of 2 rabbits were rinsed with 20 ml distilled water 20 seconds after the instillation. One animal in the rinsed group died on day 3, and was replaced by another rabbit, which was treated the same way. All eyes were examined 1 hour and 1, 2, and 3 days post application. Scoring was done according to the Draize-method.

Results
The test substance caused redness, swelling and discharge in the eyes of all animals after 1 hour. By day 2, clearing was noted in 3 of 4 animals. All treated eyes were clear at day 3. Rinsed eyes were less irritated than those not rinsed. The test substance was not classified as irritant in the rabbit eye irritation test.

Ref.: 19 (subm. II)

Comment
The SCCS considers the test substance as slightly irritant to rabbit eye.
The study was not performed according to a guideline and was not GLP compliant.

Guideline: /
Species/strain: Pirbright White guinea pig (SPF-Quality)
Group size: 5 females
Test substance: Disperse Violet 1 (1,4-diaminoanthraquinone)
Batch: /
Purity: /
Dose: 1% in water
Administration: 0.1 ml
GLP: not in compliance
Study period: 19 – 20 September 1985

Method
0.1 ml of disperse Violet 1 dissolved in water (1%) was instilled in the conjunctival sac of 5 females Pirbright White guinea pig. Eyes reaction was recorded at 0.5, 1, 2, 3, 4, 6 and 7 days after application. Reactions scored according to the Draize method.

Results
The highest irritation index was 2.0 out of a possible maximum score of 110.

Conclusions
Under the experimental conditions used, the test substance was non-irritant.

Ref.: 18 (subm. II)

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline: OECD 429 (2002)
Species: Mouse, CBA/J
Group: 20 females
Substance: Disperse Violet 1
Batch: MB002
Purity: 97.9%
Concentrations: 0.5, 1.5, 4.5 and 9% (w/v)
Dose: 25 µl
Vehicle: DMSO
Control: 1% p-phenylenediamine
GLP: in compliance
Study period: 9 – 14 October 2002

5 groups of mouse received 25 µl of Violet 1 at concentrations 0.5%, 1.5%, 4.5% and 9% respectively on the dorsal surface of each ear. All animals received an intravenous injection (Infusion pump) of 250 µl of PBS containing 20 µCi of [^{3}H] methyl thymidine. 5 hours later the animals were sacrified, the auricular lymph node taken and weighed and a single cell suspension obtained from each animal.
The mean DPM for each group was calculated. Increases on [^{3}H]-tymidine incorporation relative to the vehicle were derived and recorded as the stimulation index (test item/vehicle ratio). A positive response is defined as 3-fold or greater increase in isotope incorporation relative to the vehicle. The EC3 value (concentration inducing a stimulation index of 3) was derived by the interpolation between two points in the stimulation index.

Results
An EC3 of 3.75% was calculated for Disperse Violet 1.

Concentration	Stimulation Index

Test item	
0.5%	1.3
1.5%	2.7
4.5%	3.1
9%	4.1
p-phenylenediamine	
1%	6.4

Conclusion
Disperse Violet 1 is a skin sensitizer.

Ref.: 20 (subm. II)

Comment
According to the grading scheme used by SCCS (SCCP/0919/05), Disperse Violet 1 is a moderate skin sensitiser.

3.3.4. Dermal / percutaneous absorption

Guideline:	OECD 428 (2004)
Tissue:	dermatomed porcine skin (ears), 300–400 µm
Group size:	12 different skin samples
Diffusion cells:	flow-through diffusion cell, 1.135 cm diameter
Skin integrity:	conductivity < 900 µS
Test substance:	Disperse Violet 1
Batch:	Ch305001 (R0015526)
Purity:	98.9% (HPLC at 254 nm) 99.2% (HPLC at 548 nm)
Test item:	basic cream formulation with 0.5% Disperse Violet 1
Doses:	20 µl
Receptor fluid:	phosphate buffered saline (PBS)
Solubility receptor fluid:	soluble in PBS (tested up to 2 µg/ml, max. concentration expected)
Stability:	stable in PBS up to 72h
Method of Analysis:	HPLC
GLP:	in compliance
Study period:	11 – 18 February 2005

The formulation with 0.5% Disperse Violet 1 was applied to 2 x 6 dermatomed porcine skin (300-400µm thick), mounted on glass flow-through diffusion cells of 1cm^2, at a dose of 20 µl/cm² which corresponds approximately to 100 µg/cm² of the hair dye. After a contact period of 60 minutes, it was washed off using 2 x 1 ml deionised water, 2x1 ml 10% shampoo solution followed by 2 x 1 ml deionised water.
Samples of the receptor fluid (phosphate buffered saline) were taken at recorded intervals over a 72h period under unoccluded conditions. PBS was slowly pumped through the receptor chambers a flow rate of 1-2 ml/h to the first 8 hours and afterwards 0.5-1.2 ml/h. The upper part of the skin (stratum corneum) was separated from the remaining skin by heat separation. Samples were analysed by HPLC. Penetration rates and distribution of disperse violet 1 in the test system were calculated.

Results
1 cell was excluded due to the poor recovery (86.5%). The results from 11 chambers are used:

	Experiment 1 (ng/cm²)					
Sample	**1**	**2**	**3**	**4**	**5**	**6**
Adsorbed	8.0	3.0	3.0	13.1	3.0	3.0
Absorbed	14.6	3.0	3.0	15.8	3.0	3.0
Penetration	67.3	42.1	62.2	42.2	94.0	56.3
Rinsings skin	73037	84488	82178	79317	81340	79740

Bioavailable (ng)	**81.9**	**45.1**	**65.2**	**58.0**	**97.0**	**59.3**
Bioavailable (%)	0.105	0.052	0.080	0.074	0.117	0.070
Recovery (%)	93.7	97.4	101.0	101.9	98.2	94.7
	Experiment 2					
Sample	**1**	**2**	**3[#]**	**4**	**5**	**6**
Adsorbed	3.0	3.0	3.0	37.0	3.0	3.0
Absorbed	3.0	3.0	3.0	20.4	3.0	3.0
Penetration	279.7	122.6	123.8	221.2	354.1	112
Rinsings skin	81336	79708	72664	72888	75812	71908
Bioavailable (ng)	**282.7**	**125.6**	**126.8**	**241.6**	**357.1**	**114.6**
Bioavailable (%)	0.346	0.168	0.151	0.311	0.454	0.145
Recovery (%)	99.8	106.6	86.5	94.2	96.8	91.3
Mean dermal Absorption Experiment 1 and 2 ± Standard deviation	**0.139 ± 0.106 µg/cm² 0.175 ± 0.134 %**					

[#] Due to poor recovery, the values of sample 3 of experiment 2 were not used for the calculation of dermal absorption

Conclusions
Under the conditions of this experiment, the mean amount considered to be systemically available was 0.14 ± 0.11 µg/cm².

Ref.: 22 (subm. II)

Comments
The two experiments resulted in significantly different mean values (0.068 and 0.224 µg/cm²). There is a high variability with a relative standard deviation (RSD) of 76%, with bioavailability values ranging from 0.045 µg/cm² to 0.357 µg/cm². Therefore, a dermal absorption of 0.36 µg/cm² (mean + 2 SD; 0.14 + 2 x 0.11 µg/cm²) of Disperse Violet 1 will be used for the calculation of the Margin of Safety.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (14 days) dermal inhalation toxicity

Taken from SCCNFP/0504/01

1,4-Diamino-anthraquinone, at a 5 % concentration in a hair dye formulation, was applied to the skin of 5 hairless mice once daily for 14 consecutive days.
Under the experimental conditions adopted, no adverse effects were observed with the tested preparation.

Ref.: 2 (subm. I)

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

Guideline:	OECD 408 (1998)
Species/strain:	rat: Wistar Crl:(WI) BR (outbred, SPF-Quality)
Group size:	80 (40 males and 40 females)
Test substance:	Disperse Violet 1
Batch:	MB002
Purity:	98.9 area% (254 nm); 94.6 weight% (NMR)
Dose:	0, 0.25, 2, 20 mg/kg bw
Vehicle:	propylene glycol
Dosing:	5 ml/kg bw
Administration:	oral gavage
Exposure:	once daily for 90 days
GLP:	in compliance

Study period: 16 March – 16 June 2004

Disperse violet 1 was prepared in propylene glycol and administered for 90 consecutive days by oral gavage to male and female Wistar rats at dose of 0.25, 2 and 20 mg/kg bw/day

No mortality occurred during the study period for any dose.
At 20 mg/kg bw diffuse midzonal/centrilobular hypertrophy of the liver was noted in most males and an increase in cholesterol levels. In females, cholesterol and triglyceride levels were also increased, and higher liver weights were found, although these findings were not supported histopatologically. A slightly lower motor activity of females was seen. Some slight changes in haematological parameters were observed at this dose in males including lower erythrocyte counts, haemoglobin and haematocrit levels and lower relative eosinophil counts and were considered to be of no toxicological significance.
At 2 mg/kg bw/day no signs of toxicity were observed, only purple discolouration of urine and staining of the body.
Some alterations in the biochemistry were considered of no toxicological significance due to the absence of a dose-related distribution.

Conclusion
A NOAEL of 2 mg/kg/day was established for this study.

Ref.: 23 (subm. II)

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 Test

Guideline:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537
Replicates:	triplicate plates in 2 independent experiments
Test substance:	Disperse Violet 1
Batch:	MB002
Purity:	100 area% (HPLC, 548 nm), 99.3 area% (HPLC, 254 nm), 94.6 weight% (NMR)
Solvent:	DMSO
Concentrations:	Experiment I: 1, 10, 30, 100, 300, 1000, 3000 and 5000 µg/plate, without and with S9-mix Experiment II: 0.3, 1, 3, 10, 30, 100, 300, 1000, 3000 and 5000 µg/plate, without and with S9-mix
Treatment:	direct plate incorporation with at least 48 h incubation without and with S9- mix
GLP:	in compliance
Study period:	23 September – 12 November 2002

Disperse Violet 1 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

Growth inhibiting or toxic effects, evident as a reduced number of spontaneous or induced revertants or absence of a normal bacterial background lawn, were observed in all tester strains without S9-mix at concentrations ≥ 100 µg/plate (TA100), ≥ 1000 µg/plate (TA98 and TA1537) and ≥ 3000 µg/plate (TA1535). With S9-mix growth inhibiting or toxic effects were noted in all tester strains at concentrations ≥ 300 µg/plate (TA102), ≥ 1000 µg/plate (TA98 and TA100) or ≥ 3000 µg/plate (TA1535 and TA1537) in all experiments.
In the presence of S9-mix Disperse Violet 1 induced significant and dose related increases in the number of revertant colonies with TA1537 and some weaker effects with TA98 and TA1535 compared to the negative control values. No clear positive results were obtained in the absence of S9 -mix. Only in tester strains TA1537 and TA98 a weak increase in the number of revertants was noted which was not dose-dependent and not reproducible.

Conclusion
Under the experimental conditions used, Disperse Violet 1 was genotoxic (mutagenic) in this gene mutation tests in bacteria.

Ref.: 24 (subm. II)

In vitro Mammalian Cell Gene Mutation Test

Guideline:	OECD 476 (1997)	
Species/strain:	L5178Y $tk^{+/-}$ mouse lymphoma cells	
Replicates:	two parallel cultures in two independent experiments	
Test substance:	KAYAKU Violet 1 WR802321	
Batch:	MB002	
Purity:	100 area% (HPLC, 548 nm), 99.3 area% (HPLC, 254 nm), 94.6 weight% (NMR)	
Solvent:	DMSO	
Concentrations:	Experiment I:	1.6, 3.3, 6.5, 13.0 and 26.0 µg/ml without and with S9-mix
	Experiment II:	1.6, 3.3, 6.5, 13.0 and 26.0 µg/ml without S9-mix
Treatment	Experiment I:	4 h treatment without and with S9-mix; expression period 72 h and selection period of 10-15 days
	Experiment II:	24 h treatment without S9-mix; expression period 48 h and selection period of 10-15 days
GLP:	in compliance	
Study period:	23 October – 14 February 2003	

KAYAKU Violet 1 WR802321 was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Test concentrations were based on the results of a pre-test on toxicity measuring relative suspension growth. In the main tests, cells were treated for 4 h (experiment I) or 24 h (experiment II) followed by an expression period of respectively 72 h or 48 h to fix the DNA damage into a stable *tk* mutation. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Toxicity was measured in the main experiments as percentage relative total growth of the treated cultures relative to the total growth of the solvent control cultures. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-experiment on toxicity increasing precipitation visible to the unaided eye occurred at 26.6 µg/ml and above without and with S9-mix at both treatment intervals. Relevant toxic effects indicated by a relative suspension growth of < 50% were observed at 106.3 µg/ml and above under heavy precipitation. Therefore the concentration range of the main experiments was limited by the solubility of KAYAKU Violet 1 WR802321. The required

level of toxicity (10-20 % survival compared to the concurrent negative controls) was not achieved in any of the experiments.
At the end of the treatment at 13 and 26 µg/ml in all cultures of both main experiments precipitation was observed by the unaided eye. No substantial and reproducible increase of the mutant frequency was observed in both experiments. A minor increase exceeding the threshold of twice the mutant frequency of the corresponding solvent control was observed in the second culture of the second experiment. Since the historical range of negative and solvent controls was not exceeded, the effect occurred at precipitating concentrations and was not reproducible in the other culture it was considered an artificial effect due to the precipitation of the test item rather than indicating a possible mutagenic effect.

Conclusion
Under the experimental conditions used, KAYAKU Violet 1 WR802321 did not induce gene mutations in this gene mutation test in mammalian cells.

Ref.: 25 (subm. II)

Comment
Due to the precipitation, the required toxicity (10-20% survival compared to the concurrent negative controls) was not reached in the experiments and consequently, this gene mutation test in mammalian is considered less reliable.

In vitro Micronucleus Test

Guideline:	draft OECD 487
Cells:	human lymphocytes from 2 healthy, non-smoking male volunteers
Replicates:	duplicates in two independent experiments
Test substance:	Disperse Violet 1
Batch:	MB002
Purity:	98.9 area% (HPLC, 254 nm), 99.2 area% (HPLC, 548 nm)
Solvent:	DMSO
Concentrations:	Experiment 1: 6.25, 12.5 and 25.0 µg/ml without S9-mix 12.5, 25.0 and 50.0 µg/ml with S9-mix Experiment 2: 6.25, 12.5 and 25.0 µg/ml without and with S9-mix
Treatment	Experiment 1: 24 h PHA followed by 20 + 28 h treatment (without S9 mix) 24 h PHA followed by 3 + 45 h treatment (with S9 mix) Experiment 2: 48 h PHA followed by 20 + 28 h treatment (without S9 mix) 48 h PHA followed by 3 + 45 h treatment (with S9 mix)
GLP:	in compliance
Study period:	20 December 2004 – 19 July 2005

Disperse Violet 1 has been investigated in the absence and presence of metabolic activation for the induction of micronuclei in cultured human lymphocytes. The suitable top concentrations for experiments 1 and 2 were based on the results of a cytotoxicity range-finding experiment measuring replication index (RI). To determine the test concentrations for micronucleus analysis in each separate experiment the RI is measured in cultures treated with 7 increasing concentrations of Disperse Violet 1. Both in the cytotoxicity range-finding experiment and in the main experiments, treatment periods were 20 h without and 3 h with S9-mix. Harvest times were 72 h (experiment 1) or 96 h (experiments 2) after the beginning of culture. The final 28 h of incubation was in the presence of cytochalasin B (at a final concentration of 6 µg/ml). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Negative and positive controls were in accordance with the draft guideline.

Results
The highest concentrations were selected on the basis of precipition noted at the end of the treatment period rather than on cytotoxicity. It was evident that there was carry-over after media change, so exposure would have been longer than the nominal times given. There was no evidence that precipitation interfered with scoring.
In the absence of S9-mix in the first experiment (24 h PHA stimulation) a biologically relevant and dose-dependent increase in the number of binucleated cells with micronuclei was not found, although two single cultures at the two highest concentrations tested showed an increase that exceeded the historical control data. Since, these results were not reproducible and the mean values fell within the range of the historical control data. they were considered biologically irrelevant. In the second experiment (48 h PHA stimulation) in the absence of metabolic activation, a biologically relevant and dose-dependent increase in the number of binucleated cells with micronuclei compared to concurrent control values was observed.
In the presence of metabolic activation both in the first experiment (24 h PHA stimulation) and the second experiment (48 h PHA stimulation), a biologically relevant and dose-dependent increase in the number of binucleated cells with micronuclei compared to concurrent control values was not observed.

Conclusion
Under the experimental conditions used, Disperse Violet 1 induced an increase in the number of cells with micronuclei and, consequently, is genotoxic (clastogenic and/or aneugenic) in human lymphocytes.

Ref.: 26 (subm. II)

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474 (1997)
Species/strain:	mouse, NMRI
Group size:	5 mice/sex/group
Test substance:	KAYAKU Violet 1 WR802321
Batch:	MB002
Purity:	100 area% (HPLC, 548 nm), 99.3 area% (HPLC, 254 nm), 94.6 weight% (NMR)
Vehicle:	1% carboxymethylcellulose (CMC)
Dose level:	437.5, 875 and 1750 mg/kg bw
Route:	intraperitoneal
Sacrifice times:	24 or 48 hours (highest dose only)
GLP:	in compliance
Study period:	15 October – 14 November 2002

KAYAKU Violet 1 WR802321 has been investigated for the induction of micronuclei in bone marrow cells of mice. Test concentrations were based on acute toxicity in a pre-test with 2 animals per sex/group, measured at various intervals around 1 to 48 h after treatment. In the main experiment mice were exposed to single *i.p*. doses of 0, 437.5, 875 and 1750 mg/kg bw. 24 h or 48 h (highest dose only) after dosing bone marrow cells were collected. The animals of the highest dose group were examined for acute toxic symptoms 1, 2-4, 6 and 24 h after start of treatment. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/TE).
Bone marrow preparations were stained with May-Grünwald/Giemsa and examined microscopically for the PCE/TE ratio and micronuclei. Negative and positive controls were in accordance with the OECD guideline.

Results

In the high dose animals, reduced motor activity, abdominal position, eyelid closure, ruffled fur, violet urine and light blue skin colour was noted from KAYAKU Violet 1 WR802321 administration until sacrifice. No cases of death occurred.
The PCE/TE ratio was not affected by KAYAKU Violet 1 WR802321 at any test concentration or sampling time as compared to the PCE/TE ratio in the vehicle control. Thus, no clear cytotoxic effect in the bone marrow was noted. The clinical signs, the occurrence of discoloured urine and the appearance of blue skin demonstrated that the item was systemically distributed and bioavailable.
Biological relevant increases in the number of micronucleated PCEs compared to the concurrent vehicle controls were not found at any dose tested, neither 24 or 48 h after treatment and neither for male and females.

Conclusion
Under the experimental conditions used, KAYAKU Violet 1 WR802321 did not induce an increase in the number of bone marrow cells with micronuclei in treated mice and, consequently, KAYAKU Violet 1 WR802321 is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 27 (subm. II)

In vivo unscheduled DNA synthesis (UDS) test

Guideline:	OECD 486
Species/strain:	male Wistar HsdCpb: WU (SPF) rats
Group size:	4 rats per dose
Test substance:	Disperse Violet 1
Batch:	Arysta, charge: Nippon Kayaku CO, LTD lot MB002
Purity:	98.9 area% at 254 nm (by HPLC), 99.2 area% at 548 nm (by HPLC),
Dose level:	0, 1000 and 2000 mg/kg bw
Route:	oral gavage
Vehicle:	30% DMSO and 70% PEG 400
Sacrifice times:	4 h and 16 h after dosing
GLP:	in compliance
Study period:	29 July 2008 – 9 October 2008

Disperse Violet 1 was investigated for the induction of unscheduled DNA synthesis (UDS) in hepatocytes of rats. Test concentrations were based on acute toxicity in a pre-experiment test with 2 animals per sex/group dosed up to the top dose recommended by the OECD guideline. The animals were treated orally and examined at intervals of 1, 2-4, 6 and 24 h after administration of the test item. In the main experiment the highest dose was the recommended top dose (2000 mg/kg bw). The animals received Disperse Violet 1 once and were examined for acute toxic symptoms at intervals of approximately 1, 2, 4, 6 and 16 h (16 h treatment group only) after administration of the test item. Cell viability was determined by the trypan blue dye exclusion method.
Hepatocytes for UDS analysis were collected 4 h and 16 h after administration of Disperse Violet 1. At least 90 minutes after plating the cells were incubated for 4 h with 5 µCi/ml ^{3}H-thymidine (specific activity 20 Ci/mmol) followed by overnight incubation with unlabelled thymidine. Evaluation of autoradiography was done after 14 days.
UDS was reported as nuclear grain counts, cytoplasmic grain counts and net nuclear grain counts (nuclear minus cytoplasmic grains). Increased net grains should be based on enhanced nuclear counts rather than on decreased cytoplasmic counts. The mean and percentage of cells in repair (defined as cells with a net grain count of at least +5) was reported separately for each animal. Unscheduled synthesis was determined in 50 randomly selected hepatocytes/slide on 2 replicate slides per rat. Negative and positive controls were in accordance with the OECD guideline.

Results

In the pre-experiment mortality was observed in one female rat immediately after application due to the application process. Both in the pre- and main experiment, all remaining animals showed reduction in spontaneous activity, ruffled fur and from 2 h after application blue urine. Cell viability after hepatocyte isolation was not substantially affected due to the *in vivo* treatment with Disperse Violet 1 at any of the treatment periods or dose groups (≥ 70%). The systemic distribution of Disperse Violet 1 and the bioavailability in the target tissue could be demonstrated by the observation of discoloured urine in the treated rats.
Neither a biological relevant increase in mean net nuclear grain count nor in the percentage of cells in repair as compared to the untreated control was found in hepatocytes of any treated animal both for the 4 h and the 16 h treatment time.

Conclusion
Under the experimental conditions used , Disperse Violet 1 did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in the *in vivo* UDS test.

Ref.: 33 (subm. II)

Taken from SCCNFP/0504/01

Dominant Lethal study

Guideline:	/
Species/strain:	rat, Sprague Dawley (TAC:N (SD) FBR)
Group size:	10 males and females per group
Test substance:	Disperse Violet 1
Batch:	SO 110787
Purity:	34.9 % Disperse Violet 1, 3.2 % Disperse red 15 and unknown
Vehicle:	diet
Dose level:	0.02, 0.06 and 0.20 %
Route:	oral, with the diet
GLP:	in compliance
Study period:	February 1990 - September 1990

Disperse Violet 1 was administered in the diet to groups of 40 male and 45-55 female Sprague Dawley rats at levels of 0, 0.02, 0.06 and 0.2%. Dose setting was based on the findings noted in a pilot study with diets containing 0.125, 0.25 and 0.4 % Disperse Violet 1. Test diets were analysed periodically to confirm stability, homogeneity and accuracy of formulation of the diet mix.
The males, treated until week 19 with the test diet, were switched to the control diet and randomly selected for two separate matings with 2 untreated virgin females each. After mating, females were observed daily and weighed on days 0, 12 and 17 of gestation. Females were killed on day 17 of gestation and the uterine content evaluated. Males were maintained on control diet and sacrificed after 28 weeks.

Results
There was no evidence of a dominant lethal effect in male rats that had been treated for 19 weeks and switched to control diets before mating with untreated virgin females.
No effects were noted for the first mating. In the second mating, the total number of resorptions was increased in the mid dose group and the number of dams with resorptions was increased in the low and mid dose groups. However, for the high dose group no differences compared to the control were noted. Due to the lack of a dose-dependency, the noted differences to the concurrent control at the mid and low doses were not considered as an adverse effect, but as biological variations. This conclusion is supported by the fact, that a comparatively low post-implantation loss was noted for the concurrent control group.

Conclusion
Under the experimental conditions used, Disperse Violet 1 did not induce dominant lethal mutations and, consequently, Disperse Violet 1 is not genotoxic in this dominant lethal assay.

Ref.: 29 (Subm. II)

Comment
Only 34.9% of the test item consists of Disperse Violet 1; the remaining of the test item includes Disperse red 15 (3.2%) and unknown compounds (> 60%). Consequently, since this information about the purity/specification of the test item is poor, the value of this dominant lethal test is limited.

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

Taken from SCCNFP/0504/01, for evaluation of dominant-lethal study part see 3.3.6.2.

The sub-chronic toxicity, reproductive effects and mutagenic potential (dominant lethal) of a hair dye formulation containing 34.9 - 41.4% 1,4-Diamino-anthraquinone (C64) and 2.22 - 3.2% 1-amino-4-hydroxyanthraquinone (C61) were evaluated in groups of 40 male and 45 or 55 female Sprague Dawley rats. They were fed with the preparation at the dietary levels of 0.02, 0.06 and 0.2% for up to six months. Clinical chemistry, haematology and histopathology studies were performed in subgroups of 10 males and 10 females after 13 and 27 weeks. After 15 weeks, 25 females per group were mated to untreated males in a teratology study. After 19 weeks, 20 males per group were removed from the test diets and mated on two separate occasions with two untreated virgin females in a dominant lethal mutagenicity study. Ten females from the mid and high levels were removed from the test diet at the same time. These males and females remained untreated until they were killed at the end of the study, together with animals that had been maintained on the test diets for the entire period.

One female at mid dose level died during the study and three rats (two high dose, one low dose), were sacrificed in a moribund condition. Discoloured violet urine was observed in all dye treated rats. The high dose level males gained significantly less weight than the controls during the onset of the study and comparatively to the high level females. Hair loss was recorded in high dose group female and in the control group. Regrowth was seen following cessation of treatment.
Statistically significant increase in liver and kidney weights were observed in various treated groups. Slight increase in cholesterol in high dose males and treated females was noted.
Histomorphologic alterations were observed in the liver at the mid and high level and in the kidney at the high dose level. Hepatic lesions seen at the high level consisted of hepatocytomegaly, principally in the centrilobular area, minimal to moderate deposition of pigment, Kupffer cell proliferation and necrosis and vacuolation of hepatocytes. Renal changes observed at the high dose level consisted of minimal brown pigment in the tubules and changes characteristic of chronic nephropathy were more frequent and more severe than those seen in control rats. Renal and hepatic effects were generally more marked in males. Concerning teratology, one malformed foetus was found in each of the control and treated groups, these observations were not dose related and not considered to be treatment related by investigators. The observed external and skeletal variations were similar across groups. There were statistically significant increases of the numbers of dams

with resorptions at the low and mid level in the second mating of the dominant lethal study. However, there were no increases in non-viable foetuses at the high dose level, nor statistically significant effects at any dosage level in the first mating.

Based on these results, the test preparation was considered by investigators not teratogenic or foetotoxic.

Ref.: 29 (Subm. II)

3.3.8.2. Teratogenicity

Taken from SCCNFP/0504/01

1,4-Diamino-anthraquinone was administered by gavage to pregnant female Sprague Dawley rats on day 6 through 15 of gestation at the dose levels of 10, 40 and 160 mg/kg bw/day according to the OECD N°414. A control group was administered with the vehicle only, a 0.5% aqueous swelling of Na-carboxymethylcellulose (CMC).

Pregnant animals were killed on day 20 of gestation; visceral and skeletal malformations were recorded on the foetuses. No female mortality was recorded during the study. However, significant maternal toxicity was observed: decrease of body weight gain and food consumption from day 6 to 11. At the external examination, six foetuses of one dam in the high dose group had severe general subcutaneous oedema in connection with discoloured placentae. At the skeletal examinations, a slightly higher incidence of lumbar rudimentary ribs in foetuses of all dosed groups was noted. At the visceral examination, severe general subcutaneous oedema with corresponding hydrocephalus and diaphragmatic herniation in foetuses of high dose group were observed. These observations were not considered by investigators to be a teratogenic effect but secondary to maternal toxicity. None of the effects recorded were considered as teratogenic.

Conclusion

Based on the findings noted in dams (reduced food intake and body weight gain) and foetuses (subcutaneous oedema) at 160 mg/kg bw, a no adverse effect level (NOAEL) of 40 mg/kg bw for both maternal and fetal toxicity was deduced from this study.

Ref.: 28 (Subm. II)

3.3.9. Toxicokinetics

Guideline:	/
Species/strain:	human intestinal epithelial cell line TC-7
Replicates:	two independent experiments
Test substance:	Disperse Violet 1
Batch:	MB002
Purity:	98.9 area% (HPLC, 254 nm), 99.2 area% (HPLC, 548 nm)
Concentration:	10 µM in HBSS (Hank's balanced salt solution), 1% DMSO
GLP:	not in compliance
Study period:	4 April – 2 May 2005

The bioavailability of Disperse Violet 1 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 x 10^{-6} cm/sec and a $P_{app} \geq 20$ x

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 ranged from 73 to 162 % (mean of 2 values) while for Disperse Violet 1 the recovery was only 10 % . The permeability figures for the reference substances propranolol (two experiments with mean P_{app} = 58.2 and 48.0 x 10-6 cm/sec), a high permeability reference compound with 90 % absorption in humans, and ranitidine (two experiments with mean P_{app} = 0.2 and <0.1 x 10^{-6} cm/sec), known to be absorbed at about 50 % in humans, were within the acceptance range of 20 - 60 x 10^{-6} cm/sec and 0 - 2 x 10^{-6} cm/sec, respectively and demonstrated the validity of the assay.
For Disperse Violet 1 a P_{app} of 33.3 x 10^{-6} cm/sec (30.62 and 36.05 x 10^{-6} cm/sec) was determined and thus the test substance was classified to be of high permeability, indicating a good absorption from the gastrointestinal tract.

Conclusion
A mean permeability in human intestinal epithelial (TC-7) cells of 33.3 x 10^{-6} cm/sec was obtained with Disperse Violet 1 which classifies the test item to be of high permeability. As the absorption across the intestinal epithelium is considered to be the limiting factor of the uptake through the gastro-intestinal tract, the high permeability observed in this assay indicates a good absorption of Disperse Violet 1 after oral administration.

Ref.: 30 (Subm. II)

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. Due to the low recovery of Disperse Violet 1 the generated data is considered of questionable value for the estimation of the bioavailability after oral administration.

3.3.10. Photo-induced toxicity

Taken from SCCNFP/0504/01

Photo-sensitisation

Guideline:	none
Species/strain:	Hartley albino guinea pigs
Group size:	8 male and 8 female
Test substance:	Disperse violet 1
Batch:	CN2121085, TSL no. 86-08
Dose:	10% for induction and 5% for challenge in a vehicle composed of 80% DAE433 (40% dimethylacetamide, 30% acetone, 30% ethanol) and 20% physiological saline
GLP:	study not in compliance

The minimal erythemal dose (MED) for UVA and UVB was determined in guinea pigs. A 150 watt xenon lamp was used to expose all animals during the study. The light scores emitted UVA (320-410 nm), UVB 280-320 nm), and visible light waves (410 nm and greater). The animals were shaved and depilated 1 day prior to induction and daily during induction and challenge.

1 ml of the test material was spread over a test site (diameter 1.8 cm) on the nuchal area on 4 consecutive days. This was repeated in 3 weeks. 1 hour after application, the animals were irradiated with ½ MED of UVA light (1 week), respectively with 1 MED of UVB light (2. and 3. week). Freund's complete adjuvant injections in physiological saline at 1:1 were done on the first and third days of the second and third application week to 4 sites around the application site. Challenge was carried out on three different sites two weeks after the last induction treatment. Each site (1.8 cm diameter) was treated with 0.1 ml of the test material for 3 consecutive days. Determination of UVB photo-contact sensitisation was done on the left lumbar area by irradiating the animals with ½ MED UVB one-hour post application. UVA photo-contact sensitisation was checked on a site below by using ½ MED UVA one-hour post application. A third site remained unirradiated for determining contact sensitisation. All sites were inspected 24 hours after each application. Musk Ambrette served as positive control in this study.

Results
The MED was 14 minutes for UVA and 90 seconds for UVB. There was no evidence of irritation at the test sites for the test substance, and it did not show any evidence of photo-allergic reaction in the guinea pigs.

Ref.: 31 (Subm. II)

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

Disperse Violet 1
(non-oxidative conditions)

Absorption through the skin	**A (µg/cm^2)**	**=**	**0.36 µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**580 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.21**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.003 mg/kg bw**
No observed adverse effect level (90-day, oral, rat)	**NOAEL**	**=**	**2.0 mg/kg bw/d**
Margin of Safety	**NOAEL / SED**	**=**	**667**

3.3.14. Discussion

Physico-chemical properties
Disperse Violet 1 is used as a hair colour in semi-permanent hair dye formulations at a maximum on-head concentration of 0.5%. Characterisation of several batches of test material was insufficient or incomplete, but the test batches used for the main studies for safety evaluation, in this opinion, were fully characterised.

The stability of Disperse Violet 1 in typical hair dye formulations is not provided.

General toxicity

In the sub-chronic toxicity study in rats, the NOAEL is 2 mg/kg bw/d. In the teratogenicity study in rats, based on the findings noted in dams (reduced food intake and body weight gain) and foetuses (subcutaneous oedema) at 160 mg/kg bw, a no adverse effect level (NOAEL) of 40 mg/kg bw/d for both maternal and foetal toxicity was deduced. No two generation reproduction study was submitted.

Irritation / sensitisation

Disperse Violet 1 was as non-irritating to guinea pig skin at a concentration of 1% in water. It was a mild irritant when applied as aqueous slurry to New Zealand white rabbit skin.
The test substance at 1% in water was classified as non-irritant in the rabbit eye irritation test.
Disperse Violet 1 is a moderate sensitiser.

Dermal absorption

The two experiments resulted in significantly different mean values (0.068 and 0.224 µg/cm²). There is a high variability with a RSD (relative standard deviation) of 76%. The bioavailability values ranged from 0.045 µg/cm² to 0.357 µg/cm². Therefore, the mean + 2 SD (0.36 µg/cm²) dermal absorption will be used for the calculation of the Margin of Safety of Disperse Violet 1.

Mutagenicity / genotoxicity

Overall, the genotoxicity of Disperse Violet 1 is sufficiently investigated for the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Disperse Violet 1 treatment resulted in the induction of gene mutations in bacteria. A significant and dose related increase in the number of revertant colonies was induced in TA1537 only whereas in 2 other strains (TA98 and TA1535) weaker effects and in the 2 remaining strains (TA100 and TA102) no effects at all were observed. In the gene mutation assay in mammalian cells at the *tk* locus of mouse lymphoma cells an induction of gene mutations was not found. In an *in vitro* micronucleus test, Disperse Violet 1 induced an increase in the number of micronucleated cells.
The positive effects found in the *in vitro* experiments could be outweighed with *in vivo* tests. Disperse Violet 1 did not induce an increase in the number of micronucleated erythrocytes in an *in vivo* bone marrow micronucleus test in mice nor UDS synthesis in an UDS test in rats.
As positive effects found *in vitro* were not confirmed in *in vivo* tests, Disperse Violet 1 can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.

Carcinogenicity

No data submitted

4. CONCLUSION

Based on the information provided, the SCCS is of the opinion that the use of Disperse Violet 1 in semi-permanent hair dye formulations at a maximum concentration of 0.5% does not pose a risk to the health of the consumer, apart from its moderate skin sensitising potential.

The fully characterised batches of Disperse Violet 1 contained up to 3% Disperse Red 15. According to Cosmetic Directive Annex II, Disperse Red 15 is only permitted as impurity in

Disperse Violet 1, but without concentration limit. The test batches of Disperse Violet 1 used for the main studies of safety evaluation contained < 1% of Disperse Red 15. Therefore, the impurity of Disperse Red 15 in Disperse Violet 1 for hair dye formulations should be <1% (w/w).

5. MINORITY OPINION

Not applicable

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