OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

BASIC VIOLET 2

COLIPA n° B115

adopted by the SCCNFP on 23 April 2004
by means of the written procedure

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is Basic Violet 2 safe for use in cosmetic products as a hair dye ingredient?

* Does the SCCNFP propose any restrictions or conditions for its use in cosmetic products?

1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.
The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1. General

Basic Violet 2 is listed as CI 42520 in Annex IV, part 1 – list of colouring agents allowed for use in cosmetic products – to Directive 76/768/EEC on cosmetic products; field of application 4: colouring agents allowed exclusively in cosmetic products intended to come into contact only briefly with the skin. Other limitations and requirements: 5 ppm maximum concentration in the finished product.

2.1.1. Primary name

Basic Violet 2 (INCI)

2.1.2. Chemical names

* 4-[(4-amino-m-tolyl)(4-imino-3-methylcyclohexa-2,5-dien-1-ylidene)methyl]-o-toluidine monohydrochloride (Cosmetic Inventory)
* 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)methylene]bis[2-methyl-, monohydrochloride (CA index name)
* Benzenamine, (4-amino-3-methylphenyl)(4-imino-3-methyl-2,5-cyclohexadien-1-ylidene)methyl]-2-methyl-, monohydrochloride

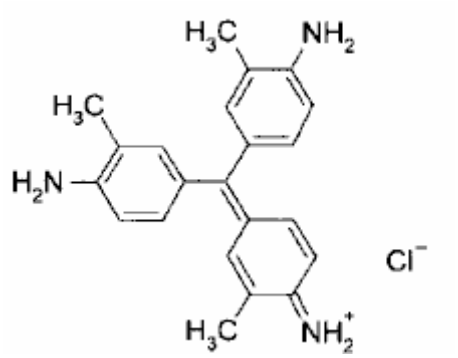
2.1.3. Trade names and abbreviations

COLIPA n°	:	B115
Trade name	:	Lowacryl Violet 2 (Lowenstein)
Synonyms	:	New Magenta

2.1.4. CAS /EINECS / Colour Index number

CAS	:	3248-91-7
EINECS	:	221-831-7
Colour Index	:	CI 42520

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{22}H_{24}N_3Cl$ (or $C_{22}H_{23}N_3.HCl$)
Mol weight : 365.91

2.1.7. Purity, composition and substance codes

Substance code : A000945
Batches used : 063-01/42-14 and 063-01/41-06

Purity : > 80% by NMR (quantitative)
Loss on drying : < 10 %
Water content : /
Ash content : < 2 %

Potential impurities
Reagents and intermediate reaction products

4-(Bis(4-amino-3-methylphenyl)methylene)-1-imino-2,5-cyclohexadiene-monohydrochloride (Carbol Fuchsin)	< 10%
4-(Bis(4-aminophenyl)methylene)-1-imino-2-methyl-2,5-cyclohexadiene-monohydrochloride (Basic Violet 14)	< 1%
1-(Bis(4-aminophenyl)methylene)-4-imino-2,5-cyclohexadiene-monohydrochloride (Basic Fuchsin)	< 100 ppm
Methyl acetate	< 3%
Aniline	< 25 ppm
o-Toluidine	< 25 ppm
p-Toluidine	< 25 ppm

Solvent residues
Detected was only methyl acetate, which originates from crystallisation (< 3%). Other solvents such as methanol, ethanol, isopropanol, n-propanol, acetone, ethyl acetate, cyclohexane, methyl ethyl ketone and monochlorobenzene were not detected.

2.1.8. Physical properties

Appearance : Dark green powder
Melting point : > 300°C *
Boiling point : 578 +/- 40°C (760 Torr, free base; calculated ACD) *
Density : /
Rel. vap. dens. : /
Vapour Press. : /
Log P_{OW} : 2.842 +/-0.652 (free base; calculated ACD) *
pKa : 6.6 +/-0.2 (most basic, calculated ACD) *

* See General Comments below

2.1.9. Solubility

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

2.1.10 Stability

Stability data provided for a common market formulation are not acceptable.

General comments on analytical and physico-chemical characterisation

* The physical properties has been calculated without indicating the method used. Furthermore, calculated values can not be accepted as estimates of the true physical constants without justification, indicating that the reported values are realistic (possible decomposition of the test substance at elevated temperatures).

* Since log P_{ow} is known to strongly depend on the pH, the reported value 2.842 +/-0.652, followed by the ambiguous specification "free base; calculated ACD", seems to correspond to an alkaline pH, well above pKa = 6.6, i.e. not related to physiological conditions and to the pH conditions of the percutaneous absorption studies.

* The reported stability data on a common market formulation are based on a single determination and comparison of the result with a "theoretical" content"; this is not an acceptable stability test.

2.2. Function and uses

Basic violet 2 is intended to be used as a non-reactive hair colouring agent (“direct” dye) in semi-permanent hair dye formulations at a maximum concentration of 0.5% in the finished cosmetic product.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Guideline	:	OECD 401 (1987) - limit test
Species/strain	:	Rat, Sprague Dawley Hsd: SD strain
Group size	:	5 males + 5 females
Batch no	:	GST 063-01/41-06
Dose	:	2000 mg/kg bw–(limit test)
Vehicle	:	0.5% CMC aqueous solution
GLP	:	in compliance

Results
Mortalities : 3 males died within 5 days of dosing
Clin. 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 no : 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.
Clin. 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.: 1

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

No data

2.3.5 Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Subchronic oral toxicity

Guideline : OECD 408 (1998)
Species/strain : rat, Sprague Dawley Hsd: SD strain
Group size : 10 males + 10 females per group
Batch no : 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
GLP : in compliance

Results
Mortality : Two high-dose females were killed in extremis on day 57. These deaths might be due to misdosing, but there was no clear evidence.
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:

Dose (mg/kg bw/d)	**3**		**10**		**30**		**dose related**
	m	**f**	**m**	**f**	**m**	**f**	
Haematology							
haematocrit	dc	-	dc	-	dc	-	yes
red blood cell count	d	d	dc	d	dc	dc	yes
haemoglobin	-	-	-	dc	-	dc	yes
MCH	-	-	ic	-	ic	-	yes
MCV	-	-	-	-	ic	-	
Clinical chemistry							
sodium	i	ic	ic	ic	ic	ic	yes (males)
calcium	ic	ic	ic	ic	ic	ic	yes (males)
potassium	ic	-	ic	-	ic	-	
cholesterol	-	-	-	-	ic	ic	
glucose	-	ic	-	i	-	ic	
ASAT activity	-	-	-	dc	dc	dc	yes
ALP activity	-	-	-	dc	-	dc	yes
Organ weights							
relative liver weight	-	-	-	-	ic	ic	
relative kidney weight	-	-	-	-	ic	ic	
relative heart weight	-	-	ic	-	ic	ic	

dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls

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
Because the changes in haematology and clinical chemistry in the low-dose group were stated to be ‘within the range of historical control data’, 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 concludes that the NOAEL is below 3 mg/kg bw/day.

Ref.: 2

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No data

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

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
Purity	:	94%
Batch no	:	GST 063-01/41-06
Dose level	:	0.5 g (4h contact under semi-occlusion)
GLP	:	in compliance

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
Since the mean irritation scores 24 to 72 hours after application were below the thresholds defined in Commission Directive 2001/59/EC, classification of the test compound as to its skin irritating properties is not required.

Ref.: 3

Comments
Assessment of skin irritation with colorants as test substances is always difficult due to the staining of the test sites and thus masking of possible erythema. Therefore, it is unfortunate that the observation period was not made longer than 72h, at least until the staining had disappeared (normal observation period is 14 days). Nevertheless, there is no reason to ask for a repetition of the skin irritation study.

2.4.2. Irritation (mucous membranes)

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
Purity	:	94%
Batch no	:	GST 063-01/41-06
Dose level	:	100 mg
GLP	:	in compliance

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 has to be labelled "R41: Risk of serious damage to eyes. " according to Commission Directive 2001/59/EC.

Ref.: 4.1

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
Purity	:	94%
Batch no	:	GST 063-01/41-06
Dose level	:	100 mg

GLP : in compliance

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.: 4.2

2.5. Sensitisation

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
Purity	:	94%
Batch no	:	GST 063-01/41-06
Dose levels	:	intradermal injection: 1% solution,
dermal induction	:	50% solution,
dermal challenge	:	50% solution
		(preliminary screening study available)
GLP	:	in compliance

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. The test is inconclusive.
Ref.: 5.1

Buehler test

Guideline	:	OECD 406 (1992). No justification is given for the performance of the 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
Purity	:	94%
Batch no	:	GST 063-01/41-06
Dose levels	:	dermal inductions: 75% solution, dermal challenge: 50% solution (preliminary screening study available)
GLP	:	in compliance

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
The test is inconclusive.

Ref.: 5.1

2.6. Teratogenicity

Guideline	:	OECD 414 (1983)
Species/strain	:	rat, Sprague Dawley Hsd: SD strain
Group size	:	24 females (mated) per group
Batch no	:	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

Results

Clin. 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 F_0	:	final body weight, uterus weight and corrected body weight were decreased 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.: 6

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Guideline	:	OECD 428 (1995)
Test system	:	Full thickness pig skin (1000 μm), 5 samples. No justification for the use of full thickness skin is given.
Contact time	:	30 minutes
Test substance	:	Basic Violet 2, used at 0.2% in a hair dye formulation (composition not stated)
Control	:	"neutral gel" (composition not stated)
Purity	:	94%
Batch no	:	GST 063-01/41-06
Application	:	100 mg/cm².

Receptor fluid : 0.14M NaCl, 2mM K_2HPO_4, 0.4mM KH_2PO_4, 100 IU penicillin/ml, 97µg streptomycin/ml, 3% ethanol
GLP : in compliance

The cutaneous absorption of Basic Violet 2 was determined in a representative hair dye formulation containing 0.2% of the test substance using pig full thickness skin samples *in vitro*. A dose of 100 mg formulation/cm² was applied on skin samples (200 µg Basic Violet *2*/cm² pig skin) for 30 minutes and subsequently rinsed off with water and shampoo. After 24 hours, the amount of the test substance was determined in the receptor fluid, in the skin extracts (epidermis and upper dermis separated) and in the rinsing solution using HPLC analysis.

Results
The amount of Basic Violet 2 in the receptor fluid was below the limit of detection of 0.0013µg/cm² (or 0.0006% of the applied dose). Correspondingly, the amount of 0.0013µg/cm² was regarded as to have passed the skin barrier during the experimental period of 24 hours. The concentrations of Basic Violet 2 detected in the separated skin layers were 0.24 ± 0.11 µg/cm² (or 0.120 ± 0.055%) in the epidermis (upper skin), and 0.003 ± 0.001 µg/cm² (or 0.001 ± 0.001%) in the upper dermis (lower skin). A total recovery of 90.89 ± 0.77% was calculated, including the amount of test substance in the rinsing solution (181.54 ± 1.47 µg/cm² or 90.77 ± 0.74%).

Conclusion
The content of Basic Violet 2 in the upper dermis (lower skin) is 80 fold lower compared to the concentration in the epidermis (upper skin) indicating that the part of the test item which remains on or in the skin after the washing steps stays mainly on or in the epidermis and that there is no measurable delivery into the receptor fluid. Thus, a dermal penetration rate of 0.0043 µg/cm²/24h (receptor fluid + upper dermis) was considered as bioavailable.
For the worst case assumption the content of the test item found in the epidermis was added to consider any possible retention of the test item in the epidermis that might be removed into the systemic compartment resulting in a maximum dermal penetration rate of 0.244 µg/cm²/24h.

Ref.: 7

Comments:

- The exact composition of the hair dye formulation in which Basic Violet 2 is incorporated, is not stated, neither is the composition of the "neutral gel" used as control.
- Basic Violet 2 was only tested at 0.2%, while the intended use concentration is 0.5%
- The justification for the use of full thickness skin is not given
- Only 5 skin samples have been used.
- The dosage was extremely high, being 100 mg/cm² instead of 1 - 5 mg/cm². In such infinite dose percutaneous absorption studies, the obtained results expressed as a percentage, are of no value for any calculation.
- The choice of the receptor fluid and the solubility of Basic Violet 2 in the receptor fluid are not documented
- An experiment with higher concentrations of the test substance is mentioned, but not included in the data package. Viewing the fact that the intended use concentration is higher than the one that has been tested, this is crucial information and should be given.

For the above reasons, the percutaneous absorption study cannot be considered acceptable.

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Reverse Mutation Testing Using Bacteria

Guideline	:	OECD 471 (1997)
Species/Strain	:	*Salmonella typhimurium* TA1535, TA1537, TA98, TA100 and TA102
Doses	:	1st exp.: 1-10-100-300-1000-5 000 µg/plate 2nd exp.: 3-10-30-100-300-1000-3000 µg/plate.
Metabolic Act.	:	S9 from rat liver homogenate (rats were treated with Aroclor 1254)
Replicate	:	2 experiments
Positive controls	:	According to OECD/471 Guideline
Test substance	:	Basic Violet 2
Batch No.	:	GST063-01/41-06 (05.06.2000) (Stable at room temperature)
Purity	:	HPLC 94.2% NMR: 96.8%;
GLP	:	in compliance.

Results
Toxicity was observed for several strains in the first experiment:

* in the absence of S9:

TA98 (1000 and 5000 µg)
TA100 (100, 1000 and 5000 µg)
TA1535 (100, 1000 and 5000 µg)
TA1537 (100, 1000 and 5000 µg)
TA102 (1000 and 5000 µg)

* in the presence of S9:

TA98 (5000 µg)
TA100 (100, 1000 and 5000 µg)
TA1535 (100, 1000 and 5000 µg)
TA1537 (1000 and 5000 µg)
TA102 (1000 and 5000 µg)

This toxicity was observed also in the second experiment.

Evaluation
An increase in the number of revertants was observed on TA102 strain only in the presence of S9, at non toxic doses: however, the effect never reached a factor of 2 compared to the control. This effect was not considered biologically relevant, or related to the induced dose. The study must be considered inadequate for the evaluation of this substance, because of the extended toxicity observed which made impossible an adequate evaluation of the intrinsic mutagenic effect of this substance on *Salmonella typhimurium* cells.

Conclusions
The study is considered inadequate.

Ref.: 8.1

***In Vitro* Mammalian Cell Gene Mutation Test**

Test Substance : Basic Violet 2

A summary is reported. The study is not available.

Ref.: 8.2

2.8.2 Mutagenicity/Genotoxicity *in vivo*

***In vivo* Mammalian Erythrocyte Micronucleus Test**

Guideline	:	OECD 474 (1997)
Species/Strain	:	Swiss CD-1 Mice (males and females)
Group size	:	5 males /5 females / group dosed
Test substance	:	Basic Violet 2
Batch No.	:	GST 063-01/41-06
Purity	:	HPLC: 94%. Substance received on 31.08.1999, stored at room temperature in the dark. Dosing performed on 18.01.2000
Dose level	:	toxicity test (48h observation): 2000. 1000, 500 mg/kg; (24h observation): 250, 125, 62.5 mg/kg; (48h observation): 31.3, 15.6, 7.81, 3.91 mg/kg. Final study: 3.00, 6.00, 12.00 mg/kg (24 and 48 h observation)
Positive control	:	Mitomycin C: 2.00 mg/kg
Negative control	:	Distilled water
Administration	:	Intraperitoneal injection (once)
Sacrifice time	:	24 and 48h
GLP	:	in compliance

Results
Toxicity Study: 3 toxicity studies were performed, in order to identify the doses to be used in the main study.
Assay 1 (2 males and 2 females); 50% survived animals at the dose of 500 mg/kg
Assay 2: (2 males and 2 females) 50% survived animals at the dose of 62.5 mg/kg
Assay 3: (2 males and 2 females) all animals survived at the dose of 31.3, 15.6, 7.81 and 3.91 mg/kg after 48 hours. No signs of reaction were observed.

Main assay
24 hours of treatment: a reduction in the PCE/(PCE+NCE) ratio was observed for both sexes.
48 hours of treatment: only animals tested with 12.00 mg/kg were analysed; 1 50% reduction of the PCE/(PCE+NCE) ratio was observed.
Mitomycin C produced also a reduction in the PCE/(PCE+NCE) ratio after 24 hours of treatment.
There were no differences between the percentages of MN in control, 24 hours and 48 hours treated animals.
Mitomycin C induced 24.5% of MN over 0.9/1.1% of the control.

Evaluation
The substance administered by intraperitoneal injection to male and female Swiss CD-1 mice did not induce an increase in the MN percentages over the untreated animals 24 and 48 hours after treatment.

No justification of the chosen route of administration was given, as requested by the Guideline.

Conclusion
Basic Violet 2 is considered non-mutagenic in this test. Under the test condition, Basic Violet 2 did not produce micronuclei in the immature erythrocytes of mice (OECD Guideline).
The toxicity observed in the bone marrow cells of treated animals indicates that the substance had reached the target cells.

Ref.: 9

2.9. Carcinogenicity

No data

2.10. Special investigations

No data

2.11. Safety evaluation

Not applicable

2.12. Conclusions

The reported stability data on a common market formulation are based on a single determination and comparison of the result with a "theoretical" content"; this is not an acceptable stability test.

The oral LD50 in rats exceeded 2000 mg/kg bw. In an oral 13 week toxicity study, the NOAEL was below 3 mg/kg bw/day.
Maternal toxicity and a delay of foetal development were observed at 50 mg/kg bw/day. There was no evidence of teratogenic effects. The NOAEL for maternal and developmental effects was 10 mg/kg bw/day.

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.

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 QAU 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 QAU statements are dated 3 to 4 years after performance of the tests. Nevertheless, the studies are accepted for evaluation.

The percutaneous absorption study was considered inadequate.

Basic Violet 2 has been tested *in vitro* on *Salmonella typhimurium* strains and *in vivo* on mice, for the induction of Micronuclei (MN).
The study presented for the *Salmonella typhimurium* was inadequate.
The study presented for the induction of MN on mice was adequate: the substance did not produce chromosomal structural and/or numerical abnormalities *in vivo*.

The data provided are not sufficient to draw a conclusion on the mutagenic/genotoxic potential of the test substance.

2.13. References

1. Basic Violet 2 Acute oral toxicity study in the rat, RTC (Research Toxicology Centre, Roma), 7121/T/213/99, October 02, 2002
2. Basic Violet 2 13 Week oral toxicity study in rats, RTC (Research Toxicology Centre, Roma), 7128/T/301/2001, December 20, 2002
3. Basic Violet 2 Acute dermal irritation study in the rabbit, RTC (Research Toxicology Centre, Roma), 7123/T/206/99, May 27, 2003
4.1. Basic Violet 2 Acute eye irritation study in the rabbit, RTC (Research Toxicology Centre, Roma), 7124/T/215/99, February 18, 2003
4.2. 1% Aqueous Solution of Basic Violet 2, Acute eye irritation study in the rabbit, RTC (Research Toxicology Centre, Roma), 7122/T/049/2000, September 18, 2002
5.1 Basic Violet 2 Delayed dermal sensitisation study in the guinea pig (Magnusson and Kligman Test), Research Toxicology Centre, Roma, 7125/T/223/99, 18.2.2003
5.2 Basic Violet 2 Delayed dermal sensitisation study in the guinea pig (Buehler Test), RTC (Research Toxicology Centre, Roma), 7126/T/224/99, March 06, 2003
6. Basic Violet 2 Oral Teratogenicity study in rats, RTC (Research Toxicology Centre, Roma), 7130/T/173/2000, July 05, 2001
7. Cutaneous Absorption of "Basic Violet 2" in a hair dye formulation through pig skin in vitro, Cosmital SA, Marly, KP031, September 30, 1999
8.1 Assessment of the potential mutagenicity of Basic Violet 2 in the Ames Reversion Assay with Salmonella Typhimurium, Cosmital SA, Marly, AT 738, August 02, 2000
8.2 Basic Violet 2 Mutation in L5178Y TK+/- Mouse Lymphoma Cells (Fluctuation Method), RTC (Research Toxicology Centre, Roma), 7281/M/01500
9 Basic Violet 2 Micronucleus Test, Research Toxicology Centre, Roma, 7280-M-00800
10.1 Identity and Purity Test of Basic Violet 2. Wella AG, G1999/027, February 03, 2000
10.2 Identity, Stability and Purity Test of Basic Violet 2. Wella AG, G1999/021, 28.8.1999
15. Stability in formulation; Sept. 22, 2003 Wella AG; D-64274 Darmstadt

References provided upon request

11. Basic Violet 2 28 Day (preliminary) oral toxicity study in the rat, RTC (Research Toxicology Centre, Roma), 7127/T/089/200, January 31, 2003
12. Basic Violet 2 Preliminary oral teratogenicity study in rats, RTC (Research Toxicology Centre, Roma), 7129/T/256/99, July 05, 20001
13. Cutaneous Absorption of "Basic Violet 2" in a hair dye formulation through pig skin in vitro, Short report (non-GLP) on an additional experiment with 1 % "Basic Violet 2" Cosmital SA, Marly, May 30, 2003

14. In vitro Transformation of Syrian Hamster Embryo (SHE) Cells by 7- Day Exposure to TM#2614, Covance Laboratories Inc. (Covance), Vienna, 22127-0-485R, 17.1.2003

3. Opinion of the SCCNFP

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.