Scientific Committee on Consumer Safety

SCCS

OPINION ON

HC Blue n° 15

COLIPA n° C182

The SCCS adopted this opinion at its 14th plenary meeting

of 27 March 2012

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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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 HC Blue n° 15, with the chemical name 4-[(2,6-dichlorophenyl)(4-imino-3,5-dimethyl-2,5-cyclohexadien-1-ylidene)methyl]-2,6-dimethylbenzenamine phosphate (1:1), was submitted September 2003 by COLIPA[1].

The Scientific Committee on Consumer Products and Non Food Products intended for Consumers (SCCNFP) adopted on 23 April 2004 the opinion (SCCNFP/0793/04) with 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, including data on stability.*

* *data on the genotoxicity/mutagenicity following the relevant SCCNFP-opinions and in accordance with the Notes of Guidance".*

According to the current update to submission I, submitted by COLIPA in July 2005, HC Blue n° 15 is used as a as a direct dye in oxidative and non-oxidative hair colouring products. The final concentration on the scalp can be up to 0.2%.

Submission 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://europa.eu.int/comm/enterprise/cosmetics/doc/hairdyestrategyinternet.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 HC Blue n° 15 safe for the consumers, when used as a direct dye in any hair dye formulations at a maximum concentration on the scalp of 0.2% taking into account the scientific data provided?*

2. *Does the SCCS recommend any restrictions with regard to the use of HC Blue n° 15 in any 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

HC Blue n° 15 (INCI)

Note: this compound is neither in the EU inventory, nor in the CTFA Dictionary.

3.1.1.2. Chemical names

Phosphoric acid compound with 4-[(2,6-dichlorophenyl) (4-imino-3,5-dimethyl-2,5-cyclohexadien-1-ylidene) methyl]-2,6-dimethylaniline (1:1)

Benzenamine,4-[(2,6-dichlorophenyl)(4-imino-3,5-dimethyl-2,5-cyclohexadien-1-ylidene) methyl]-2,6-dimethyl-, phosphate (1:1)

Phosphoric acid compound with 4-[(2,6-dichlorophenyl)(4-imino-3,5-dimethyl-2,5-cyclohexadien-1-ylidene)methyl]-2,6-dimethylaniline (1:1)

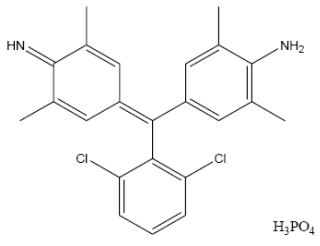
3.1.1.3. Trade names and abbreviations

Jade Blue
Jade Blue WR
802178
Basic Blue 77 phosphate
COLIPA C182
Gardex Jade Blue
WR 802178
A015892
Basic Blue 77

3.1.1.4. CAS / EC number

CAS: 74578-10-2
EC: 277-929-5

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{23}H_{22}Cl_2N_2 \cdot H_3PO_4$

3.1.2. Physical form

Reddish brown powder

3.1.3. Molecular weight

Molecular weight: 495.34 g/mol

3.1.4. Purity, composition and substance codes

Identity, purity and stability was tested in a material which was supplied by an applicant, designated as: Gardex Jade Blue; WR 802178;A015892; lot BB77-020420.

With the exception of the toxicokinetic study, all toxicological tests have been performed with lot BB77-020420. The following results are taken from the summary of the updated submission of 2005. (These results are in a rough agreement with those given in reference 1 from the submission of 2002).

The identity of the test substance was verified by MS-, IR- and UV/Vis-spectroscopy.

Purity:	HPLC (254 nm) semi-quantitative	>95% area (cation)
	NMR	42-46% w
Loss on drying:		3-7% w
Water content:		6-10% w
Sulfated ash content:		13-18%

Potential impurities:

2-Propanol:	<5% w
Starch:	25-35% w
PEG 800:	<2% w
Phosphates:	10-15% w
Boron:	<1.5%
Lead:	<20 ppm
Mercury:	<1 ppm
Arsenic:	<3 ppm
Iron:	<100 ppm

The purity of the organic cation of the test substance was tested with ^{1}H- and ^{13}C NMR. The inorganic anion was quantified with 31P-NMR.

For the NMR quantification of the cationic part of the test substance and the organic impurities dimethylaminobenzoic acid ethylester and thymol were used as internal standards.
For the NMR quantification of the anionic phosphorous component phosphonoacetic acid was used as an internal standard. Ref 1

Water was determined using Karl Fischer titration.

Starch was determined according to Kolthoff's procedure.

There are no data about the methods used for the quantification of metal impurities.

3.1.5. Impurities / accompanying contaminants

See 3.1.4.

3.1.6. Solubility

Water: approximately 61.9g/l

Due to the gelatinous agglomeration of the test substance, the solubility could not be determined accurately.

Ref.: 4

The following solubilities have been supplied with the updated submission 2005.
In the summary of this submission ref 1 has been cited as the source, but in this reference no data on solubility are given.

Water:	3.0% w/w
Acetone/water 1:1:	6.4% w/w
DMSO:	9.3%w/w

3.1.7. Partition coefficient (Log Pow)

Log Pow: 3.47 -+ 0.1

Log Pow has been determined with HPLC/DAD according to EC A.8 and OECD guideline 117.

Ref.: 6

3.1.8. Additional physical and chemical specifications

Melting point:	could not be determined; degradation at 203 °C	(EC A.1)	ref 7
Boiling point:	could not be determined; degradation at 203 °C	(EU A.2)	ref 8
Flash point:	not highly flammable	(EC A.10)	ref 12
Vapour pressure:	<1.0 exp-7 hPa (20 °C)	(EC- A.4)	ref 10
Density:	1.424g/l(20 °C)	(EC- A.3)	ref 9
Viscosity:			
pKa:	4.40		ref 5
Density:	1.424g/ml (20 °C)	(EC A.3)	ref 9

3.1.9. Homogeneity and Stability

The stability of the test substance was tested in:

DMSO 5% w/v
Acetone 0.2% w/v
Phosphate buffered solution (pH7.6)w/v

Immediately after preparation as well as 6h, 2d and 8d aliquots of the test solutions were analyzed by HPLC.
The recoveries in DMSO were 99.9 (6h), 102.2(2d) and 96.8 % (8d).
The respective recoveries in acetone were 97.6, 95.5 and 99.4%.
The respective recoveries in buffer solution were 98.0, 84.8 and 43.5%.

Stability has been tested in an common market formulation. According to submission 2005 "it was confirmed that, from the analytical point of view, after 10 month at 25 °C the dyestuff HC Blue n° 15 is stable in the tested cosmetic formulation "Strähnenhaarfarbe

Nordish Blond/89". The analyses were performed with HPLC. Quantitative results have not been supplied.

General Comments to physico-chemical characterisation

- For the toxicological tests, a batch of the test substance had been used that is sufficiently characterized with respect to identity, purity and stability.

- Data about homogeneity of the test substance or its formulations have not been supplied.

- The test substance is stable in solutions of DMSO and acetone at RT for at least 8 days. During this time interval the test substance decomposes in a buffered water solution (decrease in concentration by about 50%).In a common market formulation the test substance was stable for at least 10 month. Original data have not been supplied.

- For the determinations of physico chemical properties (3.1.8.) a batch with higher purity (90.8%: batch 906/17), than that for toxic studies, have been used. The batches used for toxic studies contained up to 35% starch.

3.2. Function and uses

HC Blue n° 15 is intended for use in oxidative hair dyes as a non-reacting component at a maximum final concentration of 0.08% to 0.10 % after mixing with 1.0 to 1.5 volumes of hydrogen peroxide preparation respectively (0.20% in the dye formulation).

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

No data submitted

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Taken from SCCNFP/0793/04

Guideline:	OECD 404 (1992)
Species/strain:	New Zealand White Rabbit, SPF
Size:	3 (both sexes)
Test item:	HC Blue n° 15
Batch:	BB77 -020420
Purity:	See Comments on analytical characterisation
Dose:	0.5g moistened with approx. 0.1 ml of purified water.
GLP:	in compliance

The primary skin irritation potential of HC Blue n° 15 was investigated by topical semi-occlusive application of 0.5 g to the intact left flank of each of three young adult New Zealand White rabbits. The duration of the treatment was four hours. The scoring of skin reactions was performed 1, 24, 48 and 72 hours, as well as 7, 10 and 14 days after removal of the dressing.

Results
Erythema could not be fully evaluated during the first 24 hours after treatment due to marked staining at the application site. However, the mean score was calculated for each animal using the data available (24, 48 and 72 hours after patch removal) for erythema/eschar grades and for oedema grades. The mean erythema/eschar scores for the three animals were 1.67, 0.00 and 1.00, respectively and the mean oedema scores were 0.33, 0.00 and 0.00, respectively.
The application of HC Blue n° 15 to the skin resulted in mild signs of irritation such as erythema, oedema and scaling. These effects were reversible and were no longer evident 10 days after treatment. A light to marked blue staining was present at the application site of all animals throughout the entire 14 day observation period. No corrosive effects were noted on the treated skin of any animal at any of the measuring intervals.

Conclusion
Based upon the referred classification criteria (Commission Directive 2001/59/EC) HC Blue n° 15 is considered to be not irritating to rabbit skin.

Ref.: 18

Comment
Under the conditions of the experiment, HC Blue n° 15 has irritant potential on rabbit skin.

3.3.2.2. Mucous membrane irritation

Taken from SCCNFP/0793/04

Study 1

Guideline:	OECD 405 (1998)
Species/strain:	New Zealand White Rabbit, SPF
Size:	1 female
Test item:	HC Blue n° 15
Batch:	BB77-020420
Purity:	See Comments on analytical characterisation
Dose:	0.1 g (undiluted)
GLP:	in compliance

The primary eye irritation potential of the test item was investigated by instillation of 0.1 g (undiluted due powder) into the left eye of a single young adult New Zealand White rabbit. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours, as well as 7 and 10 days after treatment.

Results
The instillation of the test item into the eye of a single rabbit resulted in early-onset and severe signs of ocular irritation. The treated eye was washed with NaCl, 0.9 % 24 hours after treatment.

Full assessment of the treated eye was prevented on a number of occasions due to the presence of a marked blue-green staining. However, examination of the eye 7 and 10 days after treatment revealed an opaque cornea and no light reflex in the iris. Effects observed in the conjunctivae consisted of reddening from 7 to 10 days after treatment and chemosis from 1 hour to 10 days after treatment. The maximum attainable score was achieved for both these parameters.
Discharge was also present throughout the 10 day observation period and was noted to be of a thick mucus type from the 48-hour examination to termination. A light to marked blue-green staining was present in the treated eye throughout the observation period.

Based on these results the animal was prematurely sacrificed at the request of the Study Director 10 days after treatment. For ethical reasons no further animals were treated.

Based on the referred classification criteria (Commission Directive 2001/59/EC of August 6, 2001), the test item poses a risk of serious damage to eyes.

Ref.: 20

Study 2

Guideline:	OECD 405 (1998)
Species/strain:	New Zealand White Rabbit, SPF
Size:	3 (both sexes)
Test item:	HC Blue n° 15
Batch:	BB77-020420
Purity:	95.7 to 99.7 area % (HPLC) (See Comments on analytical characterisation)
Dose:	0.1 ml of a 2% aqueous solution (pH adjusted to pH 6.30)
GLP:	in compliance

The primary eye irritation potential of the diluted test item (2% in purified water) was investigated by instillation of 0.1 ml into the left eye of each of three young adult New Zealand White rabbit. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours, as well as 7 and 10 days after test item application.

Results
The mean score was calculated across 3 scoring times (24, 48 and 72 hours after instillation) for each animal for corneal opacity, iris, redness and chemosis of the conjunctivae, separately. The individual mean scores for corneal opacity and iris were 0.00 for all three animals. The individual mean scores for the conjunctivae were 1.00, 1.00 and 0.67 for reddening and 0.00, 0.00 and 0.00 for chemosis, respectively.

The primary eye irritation score was calculated by totalling the mean cumulative scores at 24, 48 and 72 hours and then dividing the resulting total by the number of data points. The primary eye irritation score was 0.89 (max. 13).

The instillation of the test item into the eye resulted in mild, early-onset and transient ocular changes, such as reddening of the conjunctivae and sclerae, discharge and chemosis. These effects were reversible and were no longer evident 7 days after treatment, the end of the observation period for all animals. Corneal opacity due to abnormal findings was observed in the iris of any animal at any of the examinations. No corrosion was observed at any of the measuring intervals, blue staining of the treated eyes by the test item was observed in all animals at the 2-hour reading and was no longer evident 48 hours after treatment.

The test item did not induce significant or irreversible damage to the rabbit eye. Based on the referred classification criteria (Commission Directive 2001/59/EC), the diluted test item is considered to be not irritating to the rabbit eye.

Ref.: 19

Conclusion
Neat HC Blue n° 15 is irritant to mucous membranes (Ref. 20). However, at a concentration of 2% (in water) slight irritation to mucous membranes was observed (Ref. 19). The concentration of HC Blue n° 15 in the final product is 0.2 %.

3.3.3. Skin sensitisation

Taken from SCCNFP/0793/04

Guideline:	OECD 429 (2000)
Species/strain:	Mouse: CBA/J
Size:	5 females per concentration
Test item:	HC Blue n° 15
Batch:	BB77-020420
Purity:	(See Comments on analytical characterisation)
Dose:	0.5, 1.5, 3 and 5 % (w/v) in DMSO
GLP:	in compliance

HC Blue n° 15 was tested in the local lymph node assay at different concentrations (0, 0.5, 1.5, 2.0, 5.0% (w/v)) in DMSO (vehicle). On days 0, 1 and 2 the animals received 2.5 µl of the test item formulation, positive control, or vehicle control on the dorsal surface of each pinnae.
Morbidity/mortality checks were performed twice daily. Clinical examinations were performed daily. Individual body weights were recorded on days -1 and 5. All animals were sacrificed on day 5 for the assessment of cell proliferation.
No mortality was observed during the study. There were no treatment-related clinical signs.

There were no treatment-related effects on body weight or body weight gains.
Positive control (p-phenylenediamine) induced a positive response, as it elicited at least a 3-fold increase in isotope incorporation relative to the vehicle. The mean stimulation index was 3.9 at the concentration of 1%.

Results
The test substance induced a negative response, as it did not elicit a least a 3-fold increase in isotope incorporation relative to the vehicle. The mean stimulation indices were 1.0, 1.7, 2.3 and 1.9 at the concentrations of 0.5%, 1.5%, 3.0% and 5.0%, respectively.

Conclusion
Based of these results, the test substance is not a skin sensitizer under the defined experimental conditions.

Ref.: 21

Comment
The highest test concentration was too low. Therefore, sensitising potential has not been excluded.

3.3.4. Dermal / percutaneous absorption

Percutaneous absorption in vitro

Guideline:	/(Draft OECD)
Tissue:	Pig skin 1000 µm thick (male)
No. donor	/
Method:	Flow through diffusion cells; 4cm^2 surface area
No. chambers	5 (and 1 control)
Chamber integrity	Tritiated water
Test substance:	WR802178
Batch	/
Purity	/
Dose levels:	100 mg/cm² of oxidative formulation (base batch 6746 11.06.2002); 1.67mg/ cm² of the dye active principle (=1.67%).
Receptor fluid:	0.14 M NaCl, 2mM K_2HPO_4, 0.4 mM KH_2PO_4,100 IU Penicillin/ml and 97 µg Streptomycin/ml
Solubility in receptor	1.94 mg/ml
Stability	/
Analytical method:	HPLC (Detection at 606 nm); Limit of quantification <122 ng/cm^2
GLP:	In compliance
Date	September 2002

The skin penetration of WR802178was evaluated in a flow through diffusion cell system using pig skin (thickness: 1000 µm and exposure area: 4cm²). The integrity of the skin samples was demonstrated with tritiated water.

The oxidative dye formulation (100mg/cm²) equivalent to 1670µg/cm² of the dye active principle was applied on the skin surface for 30 min. Then, the skin excess was washed off with shampoo and water and left unoccluded for a 72 hour exposure period. At 16, 24, 40, 48, 64, and 72 hours, the dye content was analysed by HPLC as well as after 72 hours in the skin compartments (epidermis and upper dermis separated).

	Skin No	Integrity-Test ^3H_2O Permeation (4 hours cumulative) [% Dose]	1) Receptor fluid (72 hours cumulative) [ng/cm²]	[% Dose]	2) Epidermis (72 hours cumulative) [ng/cm²]	[% Dose]	3) Upper dermis (72 hours cumulative) [ng/cm²]	[% Dose]	4) Rinsing solution (after 30 minutes) [ng/cm²]	[% Dose]	1) + 2) +3) +4) Total [ng/cm²]	[% Dose]
Application of 1.67 mg of WR802178 in 100 mg of oxidative formulation per 1 cm² of skin	2	0.73	<122*	<0.007*	306	0.018	81	0.005	1'898'656	113.919	1'899'166	113.950
	4	0.92	<122*	<0.007*	425	0.025	88	0.005	1'868'304	112.098	1'868'940	112.136
	6	0.82	309	0.019	1'204	0.072	241	0.014	1'831'633	109.898	1'833'387	110.003
	8	0.85	129	0.008	403	0.024	38	0.002	1'801'745	108.105	1'802'314	108.139
	10	0.91	<122*	<0.007*	359	0.022	43	0.003	1'745'510	104.731	1'746'034	104.762
Control skin**	12	0.82	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Mean		**0.84**	**<161**	**<0.010**	**539**	**0.032**	**98**	**0.006**	**1'829'170**	**109.750**	**1'829'968**	**109.798**
± S.D.		0.07	83	0.005	374	0.022	83	0.005	59'408	3.564	59'432	3.566
(n)		(6)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)

*= limit of quantification; **oxidative formulation w/o WR802178

Results
The total recovery of the dye was 109.8 ± 3.6%. Most of the WR802178 applied on the skin surface was removed with the washing procedure. The amounts of WR802178 detected in the separated skin layers were: 0.539 ± 0.374 µg/cm² (0.032± 0.022% of the applied dose) in the epidermis, and 0.098 ± 0.083 µg/cm² (0.006± 0.005% of the applied dose) in the upper dermis.
The content of WR802178 in the receptor fluid was below the limit of quantification in 3 samples (122 ng/cm²); the amount considered as being present in the receptor was 0.161 ± 0.083 µg/cm² (0.010± 0.005% of the applied dose).

The total percutaneous absorption value (receptor fluid, epidermis and upper dermis) was 0.798 ± 0.480 µg/cm² (0.048± 0.029 % of the applied dose).

Ref.: 24

Comment
The experiment did not conform to guidelines. 100mg/cm² of formulation was applied. Too few chambers were used (5) and the number of donors is not stated. The purity of WR802178 is not given. The membrane thickness was inappropriate.
In view of the above, and as penetration of WR802178 is low, the amount considered as being absorbed from an oxidative hair dye formulation containing 1.67% WR802178 is considered as (mean +2SD) 1.76 µg/cm² (0.11%). This may be used in calculating the MOS.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral toxicity

Taken from SCCNFP/0793/04

Guideline:	OECD 407 (1995)
Species/strain:	Wistar rat
Group size:	10 animals (5 males & 5 females) / dose level
Observ. Period:	28 days (no recovery group included)
Test substance:	Basic Blue 77
Batch:	BB77-020420
Purity:	98.7 - 99.7%
Vehicle:	bidistilled water with 0.5 % 1,2-propylene glycol and 0.4% Plantaren 2000 UP

Dose levels: 0, 5, 15, 45 mg/kg bw/day
GLP: not in compliance

The test item was administered daily by oral gavage to SPF-bred Wistar rats of both sexes at dose levels of 5, 15 and 45 mg/kg bw/day for a period of 28 days. A control group was treated similarly with the vehicle, bidistilled water with 0.5 % 1,2-propylene glycol and 0.4% Plantaren 2000 UP, only.
The group comprised 5 animals per sex, which were sacrificed after 28 days of treatment. Clinical signs, food consumption and body weights were recorded periodically during pretest and treatment periods.
At the end of the dosing period, blood samples were withdrawn for haematology analyses. All animals were killed, prepared for necropsy and examined post mortem. Histological examinations were performed on organs and tissues from all control and high dose animals and on all gross lesions from all animals.

Results
Oral administration of the test item to Wistar rats at doses of 5, 15 and 45 mg/kg bw/day, for 28 days resulted in no mortalities, no clinical signs of toxicological relevance, no changes in food consumption or body weight and no changes in haematology parameters.

Test-related findings were

5 mg/kg bw/day:	slightly pale faeces from d11 of treatment onwards.
15 mg/kg bw/day:	blue discoloration of faeces from d6 onwards, discoloration of salivary glands, increased liver weights, coinciding with slight to minimal centrilobular hypertrophy in the liver
45 mg/kg bw/day:	blue discoloration of faeces from d3 onwards and blue discoloration of body extremities from d6, discoloration of the liver, salivary glands, thymus (or discoloured foci) and exorbital lacrimal gland; increased liver weights, coinciding with slight to minimal centrilobular hypertrophy in the liver (more severe in females than in males).

Conclusion
Based on the results of this study, the doses for the 90 days study were set on 0, 1, 4 and 15 mg/kg bw/day.

Ref.: 16

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

Taken from SCCNFP/0793/04

Guideline: OECD 408 (1998)
Species/strain: rat, HanBrl:WIST (SPF)
Group size: 20 animals (10 males & 10 females) / dose level
Observ. Period: 92 days (no recovery group included)
Test substance: Basic Blue 77
Batch: BB77-020420
Purity: 95.7 - 99.7%
Vehicle: bidistilled water with 0.5% 1,2-propylene glycol and 0.4% Plantaren 2000 UP
Dose levels: 0, 1, 4, 15 mg/kg bw/day
GLP: in compliance

The test item was administered daily by oral gavage to SPF-bred Wistar rats of both sexes at dose levels of 1, 4 and 15 mg/kg body weight/day for a period of 91/92 days. A control group was treated similarly with the vehicle only.

Clinical signs, outside cage observations, food consumption, and body weights were recorded during the pre-experiment and the main experiment. Ophthalmologic examinations were performed both at the end of the pre-experiment and the main experiment. During week 13 the animals were evaluated according to a functional observational battery, including locomotor activity and grip strength.
At the end of the period, blood samples were withdrawn for haematology and plasma chemistry analyses. Urine samples were collected for urinalyses. All animals were scarified, prepared for necropsy and examined post mortem.
Histological examinations were performed on organs and tissues from all control and high dose animals and on all gross lesions from all animals.
From the animals of the low and middle dose groups, livers (females) and hearts (males) were examined to establish a no-effect-level.

Results
Oral administration of the test item to Wistar rats at doses of 1, 4 and 15 mg/kg bw/day, for 13 weeks resulted in no test item-related deaths, no clinical signs of toxicological relevance during daily or weekly (weeks 1 to 12) observations, no changes in the parameters of the functional observational battery (including grip strength or locomotor activity), no changes in mean food consumption or body weight development, no ophthalmoscopic changes, and no changes in haematology parameters.

Test item-related findings were:

1 mg/kg bw/day:	no substance related effects noted.
4 mg/kg bw/day:	blue faeces and/or grey discoloration of fur, discoloration of salivary glands, and elevated mean sodium levels.
15 mg/kg bw/day:	blue faeces and/or grey discoloration of fur, discoloration of salivary glands, discoloration of extraorbital lacrimal glands and preputial glands (no microscopic correlation found for the discoloration), elevated mean sodium levels, decreased mean glucose levels in females, elevated mean triglyceride levels, elevated mean cholesterol and mean phospholipid levels in females (considered to be indications of possible changes in non-specific metabolic pathways in the liver), increased urinary leukocytes, increased urinary erythrocytes in females, elevated liver weights, coinciding with minimal hypertrophy of centrilobular hepatocytes, elevated kidney weights; a marginally greater incidence and severity of focal myocarditis was observed, but this was found to be more likely fortuitously than substance-related (statistically non-significant).

Conclusion
The results of this study indicate that 1 mg/kg bw/day of Basic Blue 77 was established as the no-observed-effect-level (NOEL), based upon passive effects at 4 mg/kg bw/day such as faecal discoloration, whereas 4 mg/kg bw/day was considered to be the no-observed-adverse-effect level (NOAEL), based upon various changes in clinical biochemistry, urinalyses and higher kidney weights at 15 mg/kg bw/day.

Ref.: 17

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Assay

Guideline:	OECD 471 (1997)	
Species/Strain:	*Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537	
Replicates:	triplicates in 2 independent experiments	
Test substance:	A015892	
Batch:	BB77-020420	
Purity:	38.5% (w/w) by NMR	
Vehicle:	DMSO	
Concentration:	experiment 1:	0, 1, 10, 100, 1000 and 5000 µg/plate, without and with S9-mix
	experiment 2:	0, 1 (TA102 only), 3, 10, 30, 100 and 300 µg/plate, without and with S9-mix
	experiment 3:	0, 0.3, 1, 3, 10, 30 and 100 µg/plate, with S9-mix, TA102 only
Treatment:	direct plate incorporation method with 48 h incubation without and with S9-mix	
GLP:	in compliance	
Study date:	19 June 2002 – 18 September 2002	

A015892 was tested in strains of *Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537 in the presence and in the absence of a metabolic activation system. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. The direct plate incorporation method with 48 h exposure was used. A third experiment with S9-mix was performed with TA102 only to solve the discrepancies between the results found in the first and second experiment.
The condition of the bacterial background lawn and the numbers of spontaneous revertant colonies were evaluated for evidence of toxicity; precipitation was evaluated by visual examination. Appropriate negative and positive controls were included.

Results
Growth inhibition and/or toxic effects detected as significant reductions in the number of spontaneous revertant colonies or absence of a normal bacterial background lawn were observed with S9-mix at concentrations ≥ 300 µg/plate with TA100 and TA1535 and at concentrations ≥ 1000 µg/plate with the other strains. In the absence of S9-mix toxic effects were observed at concentrations ≥ 300 µg/plate with all strains used. Consequently, only 3 evaluable concentrations/strain remained.
In both experiments, a biologically relevant increase in the number of revertant colonies was not observed with any strain used, at any concentration both without and with S9-mix.

Conclusion
Under the experimental conditions used A015892 was not genotoxic (mutagenic) in this gene mutation tests in bacteria.

Ref.: 26

Comment
Due to toxicity observed predominantly at concentrations ≥ 300 µg/plate the number of evaluable concentrations/strain is limited to 3.

***In vitro* Mammalian Cell Gene Mutation Test**

Guideline:	OECD 476 (1997)
Species/strain:	Mouse lymphoma cell line L5178Y $tk^{+/-}$
Replicates:	duplicate cultures in 2 independent experiments
Test substance:	Jade Blue WR 802178
Batch:	BB77-020420

Purity:	99.7 area % (HPLC, 600 nm) 31 % (w/w) by NMR
Vehicle:	DMSO
Concentrations:	experiment 1: 0, 1.4, 2.8, 5.5, 8.3 and 11 µg/ml without S9-mix 0, 2.8, 5.5, 11, 22 and 44 µg/ml with S9-mix experiment 2: 0, 0.4, 0.8, 1.5, 3 and 6 µg/plate without S9-mix
Treatment:	experiment 1: 4 h treatment both without and with S9-mix; expression period 72 h and a selection period of 10-15 days. experiment 2: 24 h treatment both without S9-mix; expression period 48 h and a selection period of 10-15 days.
GLP:	in compliance
Study date:	17 September 2002 – 8 November 2002

Jade Blue WR 802178 was assayed for mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of metabolic activation. The cells were tested before freezing on stock cultures for mycoplasma contamination and karyotype stability. Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-test for toxicity with a wide range of test concentrations up to 700 µg/ml, 4 h and 24 h treatment periods and both without and with S9-mix. Toxicity was evaluated by the cell density of the cultures immediately after treatment and on each day of the growth period and by calculating the suspension growth at the end of the growth period relative to the untreated controls.
In the main test, cells were treated for 4 h or 24 h followed by an expression period of 72 h or 48 h, respectively, to fix the DNA damage into a stable *tk* mutation. To discriminate between large (indicative for mutagenic effects) and small colonies (indicative for a clastogenic effect) colony sizing was performed. Toxicity was measured as total growth relative to the growth of the solvent control cultures. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-test precipitation occurred at 350 µg/ml and above both without and with metabolic activation. Relevant toxic effects were already seen at the lowest concentration of 5.5 µg/ml in the absence of metabolic activation; cell growth was completely suppressed at higher concentrations. In the presence of metabolic activation strong toxic effects occurred at 21.9 µg/ml and above.
In experiment 1 both in the absence and presence of S9-mix the appropriate level of toxicity (10-20% survival after the highest evaluable concentration) was not reached. In experiment 2 the highest concentrations tested showed the appropriate level of toxicity.
Biologically relevant increases in mutant frequency were not found following treatment with Jade Blue WR 802178 at any dose level tested, either in the absence nor presence of S9-mix in both experiments with a few exceptions. Two isolated increases in experiment 1 and one in experiment 2 exceeded the threshold of twice the mutant frequency of the background control as well as the range of the historical control values. However, since a concentration dependent increase was lacking and the positive results were not reproducible, they are considered not biologically relevant

Conclusion
Under the experimental conditions used, Jade Blue WR 802178 was considered not genotoxic (mutagenic and clastogenic) in this gene mutation assay in mouse lymphoma cells.

Ref.: 27

In vitro **micronucleus test in human lymphocytes**

Guideline:	draft OECD 487 (2004)

Cells:	human lymphocytes from 2-3 healthy, non-smoking male volunteers	
Replicates:	duplicate cultures in 2 independent experiment	
Test substance:	HC Blue n° 15 (WR 18043)	
Batch:	BB77-020420	
Purity:	99.7% (HPLC at 600 nm)	
Solvent:	DMSO	
Concentrations:	experiment 1:	3, 9 and 15 µg/ml without S9-mix 30, 50 and 60 µg/ml with S9-mix
	experiment 2:	3, 9, 12 and 15 µg/ml without S9-mix 30, 50, 60, 80 and 100 µg/ml with S9-mix
Treatment	experiment 1:	24 h PHA stimulation followed by 3 (with S9-mix) or 20 h (without S9-mix treatment; cells were harvested 72 h after culture initiation.
	experiment 2:	48 h PHA stimulation followed by 3 (with S9-mix) or 20 h (without S9-mix) treatment; cells were harvested 96 h after culture initiation.
GLP:	In compliance	
Date:	28 September 2005 – 14 November 2005	

HC Blue n° 15 has been investigated for the induction of micronuclei in cultured human lymphocytes in the absence and presence of metabolic activation. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Blood from two or three healthy, non-smoking male volunteers was used for each experiment in this study. The mitogen phytohaemagglutinin (PHA) was included in the culture medium in order to stimulate the lymphocytes to divide, and blood cultures were incubated at 37° C for 24 h or 48 h and rocked continuously.
The concentration selection for the micronucleus test was based on the cytotoxicity data from a range-finder experiment measuring replication index (RI). The top dose for micronucleus analysis was to be the one at which at least approximately 60% reduction in RI occurred or the highest dose tested. Both in the range finder and in the main test, cells were treated either for 3 h in the presence of S9-mix or 20 h in the absence of S9-mix, and sampled 48 h after start of treatment. Cytochalasin B (final concentration 6 µg/ml) was added the final 28 h before harvest. Negative and positive controls were in accordance with the OECD guideline.

Results
Measurements on post-treatment media from the range-finder experiment indicated that HC Blue n° 15 had no marked effect on osmolality and pH both in the absence and presence of S9-mix.
Treatment with HC Blue n° 15 in the absence and presence of S9-mix resulted in frequencies of binucleate cells with micronuclei which were nor different from those observed in the concurrent control cultures. The one exception was at the lowest concentration in experiment 1 where a statistically significant increase was apparent. However, the increase was small and fell well within the range of the negative control data. As such, these increases were considered to be of no biological significance.

Conclusion
Under the experimental conditions used HC Blue n° 15 did not induce an increase in cells with micronuclei and, consequently, is not genotoxic (clastogenic and/or aneugenic) in cultured human peripheral lymphocytes *in vitro*.

Ref: 28

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

Taken from SCCNFP/0793/04

***In vivo* Mammalian Erythrocytes Micronucleus Test**

Guideline:	OECD 474 (1997)
Species/strain:	NMRI mice
Group size:	5 mice/sex/group
Test substance:	Jade Blue WR 802178
Batch:	BB77-020420
Purity:	99.7 area % (HPLC, 600 nm) 31.0%(w/w) by NMR
Vehicle:	de-ionised water
Dose level:	0, 12.5, 25 and 50 mg/kg bw
Route:	i.p. injection
Sacrifice times:	24 h and 48 (highest dose only) h after injection
GLP:	in compliance
Study date:	14 August 2002 – 8 November 2002

Jade Blue WR 802178 was investigated for the induction of micronuclei in bone marrow cells of mice. Test concentrations were based on the results of a preliminary study on toxicity. Two mice per sex were treated under identical conditions as in the main test *i.p.* with doses ranging from 20 mg/kg bw up to 2000 mg/kg bw and observed for acute toxic symptoms at intervals around 1, 2-4, 6, 24, 30 and 48 h after start of treatment.
In the micronucleus test mice were treated by *i.p.* injection with 0, 12.5, 25 and 50 mg/kg bw. The mice of the high dose group were examined for acute toxic symptoms at intervals of around 1, 2-4, 6 and 24 h after treatment. Bone marrow cells were collected 24 h and 48 h (highest dose only) after dosing. 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 preliminary toxicity study all animals died at doses of 100 mg/kg bw and above; at 75 mg/kg bw still 1 male and 1 female mice died within 4 h after treatment. At doses of 50 mg/kg bw and above the mice showed clinical symptoms like reduction in spontaneous activity, abdominal position, eyelid closure, ruffled fur and apathy. Mice treated with 20 mg/kg bw still showed reduction in spontaneous activity, eyelid closure and ruffled fur but to a much lower extent. All treated mice had coloured urine. For the main experiment 50 mg/kg bw was estimated to be suitable top dose. In the micronucleus test identical clinical signs, coloured urine and additionally coloured skin were observed.
In the micronucleus test the PCE/TE ratio dose dependently decreased as compared to the mean ratio of the vehicle control, thus indicating that Jade Blue WR 802178 did exert cytotoxic effects in the bone marrow. Additionally, the skin and urine of the mice turned blue after treatment confirming systemic distribution and thus bioavailability of Jade Blue WR 802178.
Compared to the concurrent vehicle controls, a biologically relevant increase in erythrocytes with micronuclei at any preparation interval and dose level after treatment with Jade Blue WR 802178 was not observed. The mean values observed after treatment with Jade Blue WR 802178 were all below or near to the level of the concurrent control group.

Conclusion
Under the experimental conditions used Jade Blue WR 802178 did not induce a biologically relevant increase in the number of erythrocytes with micronuclei of treated mice and, consequently, Jade Blue WR 802178 is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 29

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/0793/04

Dose-range finding prenatal development toxicity study

Guideline:	/
Species/strain:	rat, HanBrl:WIST (SPF)
Group size:	5 females / dose level
Observ. Period:	21 days
Test substance:	Basic Blue 77
Batch:	BB77-020420
Purity:	95.4 - 98.7%
Dose levels:	0, 5, 15, 150 mg/kg bw/day
GLP:	/

Basic Blue 77 was administered by gavage in vehicle (1,2-propylene glycol, 50% aqueous decylglycoside and water) at daily dosages of 0, 5, 15 and 150 mg/kg bw/day to 5 mated Wistar rats per group from day 6 to 20 of gestation inclusive, using a dosing volume of 10 ml/kg bw. Dams were killed on day 21 *post coitum*, just prior to expected delivery and foetuses were removed by caesarean section for examination.

Results
All animals in the 150 mg/kg bw/day dosage group showed bluish-green skin discoloration, dark faeces and blue discoloured urine. They showed reduced food consumption, body weight loss and all died before day 5 post coitum.
At 15 mg/kg bw/day, a slightly reduced body weight gain was noted, besides ruffled fur from day 8 till days 12 or 13 post coitum.
Neither the reproduction data, nor the foetal parameters were affected by substance administration.

Conclusion
Based on the results of this study, the doses for the prenatal developmental toxicity study were set on 0, 3, 10 and 30 mg/kg bw/day.

Ref.: 22

Prenatal development toxicity study

Guideline:	OECD 414 (2001)
Species/strain:	rat, HanBrl:WIST (SPF)
Group size:	24 females / dose level
Observ. Period:	21 days
Test substance:	Basic Blue 77
Batch:	BB77-020420
Purity:	95.4 - 98.7%
Dose levels:	0, 3, 10 and 30 mg/kg bw/day
GLP:	in compliance

Basic Blue 77 was tested for its embryotoxic, foetotoxic and teratogenic potential in rats. The test item was administered by gavage in vehicle (1,2-propylene glycol, 50% aqueous decylglycoside and water) at daily dosages of 0, 3, 10 and 30 mg/kg bw to 24 mated Wistar rats per group from day 6 to 20 of gestation inclusive, using a dosing volume of 10 ml/kg bw. Dams were killed on day 21 post coitum, just prior to expected delivery and foetuses were removed by caesarean section for examination.

Results
There were no indications of an adverse effect of treatment with Basic Blue 77 on pregnant females on terms of mortality, clinical signs or necropsy observations.

Test item-related findings were:

3 mg/kg bw/day:	no adverse effects noted.
10 mg/kg bw/day:	blue discoloured content in urinary bladder, reduced food consumption (d6-d9 post coitum), slightly reduced body weight gain.
30 mg/kg bw/day:	dark discoloured faeces, blue discoloured content in urinary bladder, blue discoloured content in stomach and intestines, reduced food consumption (d6-d18 post coitum), reduced body weight gain.

No treatment-related effects on reproduction data or foetal data were observed. Mean number of implantation sites, pre- and post-implantation losses and mean number of viable foetuses per litter and group were not affected by treatment. There were no dead or aborted foetuses.
Mean sex ratios and mean foetal body weights were not affected by treatment. There were no treatment-related foetal external, visceral or skeletal (bones and cartilage) findings.

Conclusion
Based on the results of this study, the no observable adverse effect level (NOAEL) for Basic Blue 77 was considered to be 3 mg/kg bw/day for females and 30 mg/kg bw/day for foetuses.

Ref.: 23

3.3.9. Toxicokinetics

Guidelines:	OECD 417 (1984) Toxicokinetics and 427 (2004) percutaneous absorption
Species/Strain:	Rats, female, Wistar Crl: (WI) BR (outbred)
Group Sizes:	4 in ADME studies (groups 1,2, 3, 4) 6 in toxicokinetics studies (5, 6, 7, 8)
Test substance:	^{14}C-labelled HC Blue No 15 (WR18403) HC Blue No 15 (WR18403)
Batches:	Labelled CFQ13944 batch 1 (Purity: 98%) BB77-020420 (38.5% w/w NMR, *99.7 area % (HPLC, 600 nm*)
Vehicles:	**Oral:** 5.8% w/w propylene glycol, 4.7% w/w Plantaren 2000 UP (cognis trade name =50%aqueous decyl glucoside), 89.5% w/w milli-U water **Intravenous:** 0.05M phosphate buffer, ph 7.6; propylene glycol; ethanol 80:10:10(w/w/w) **Dermal:** Dimethylsulfoxide (DMSO)
Dose level:	**1 and 5** intravenous 4 mg/kg bw **2 and 6** oral 4 mg/kg bw (gavage) **3 and 7** oral 40mg/kg bw (gavage) **4 and 8** dermal 16.7 mg/ml (~5.57 mg/kg bw and 0.167 mg/cm^2), exposure 30 min
Study length:	**ADME** 96h **Toxicokinetics** oral and intravenous 72h, dermal 168 h

GLP: in compliance
Date: 2005

Based on an oral 28 d study, the intravenous dose and low oral dose were selected on the NOAEL of 4 mg/kg bw and the 10-fold higher oral dose based on increased liver weight and centrilobular hypertrophy.
The selected dermal application level was higher than that for normal usage, but was chosen for comparison of the bioavailability with the other administration routes. Exposure was for 30 min.
For the **ADME study**, in the oral and intravenous groups, urine and faeces were collected over 0-8, 8-24, 24-48. 48-72 and 72· 96 h and the animals were killed 96 hours post-dosing. In the dermal group, urine and faeces continued to be collected from 96-120, 120 – 144 and 144-168 h. Total radioactivity in urine, faeces, tissues and organs was determined. Selected urine and faeces samples were group pooled and analysed for the parent compound and metabolites.
In the **toxicokinetic** groups, blood was sampled from selected rats at 0.25, 0.5, 1, 2. 4. 6, 8, 24. 48 and 72 hour post-dosing. Total radioactivity and HC Blue No. 15 (WR 18043) equivalent concentrations were determined.

Results
No mortality was observed in the study. In the intravenous and oral dose groups, no clinical signs were observed, except for some blue/green discoloration of the urine and faeces on the day of dosing.
Oral absorption was calculated in two ways - from the urinary data and the plasma data. With the urinary data, the fractional absorption was calculated by dividing the percentage of radioactivity recovered in the urine after oral administration by the percentage of radioactivity recovered in the urine after intravenous administration. For the plasma data. absorption was calculated by dividing the dose-normalized area under the curve (AUC) after oral administration by the AUC after intravenous administration. Calculation of the fractional oral absorption from the urine data assumed that the ratio of excreted urinary radioactivity to systemically available radioactivity is constant for both oral and intravenous routes.
For the high oral dose group. an average oral absorption of 34% was seen when calculated from the urine data and 33% when calculated from the plasma data, showing comparability between both oral and intravenous routes.
For the low oral dose group, an average oral absorption of 62% was observed when calculated from the urine data and 35% when calculated with the plasma data. Since urinary excretion tends to be low, calculations based on plasma data were assumed to be more precise.
The average dermal absorption was very low, 2.65% or 0.005 mg/cm^2. The possibility remains that the quantity retained at the skin application site could become systemically available. Therefore, the dose was calculated on the absorbed fraction and the potentially systemically absorbed fraction to yield the "total potentially absorbed fraction". This was 3.10% of the applied dose or 0.006 mg/cm^2.
The plasma data indicated that oral absorption was moderately slow. with T_{max} values of 6-8 hours. Plasma concentration versus time curves of HC Blue No. 15 (WR 18043) equivalents after oral dosing were similar, with similar T_{max} values. and dose-normalised C_{max} and AUC of similar order of magnitude, suggesting linear kinetics over the dose range investigated. Apparent terminal half-lives were also similar, 29 and 20 h respectively in the low and high oral dose groups and after intravenous administration (17 hours). No toxicokinetic evaluation could be performed for the dermal group, since the concentrations were below the limit of quantification.
Faecal excretion was the most important route of ^{14}HC Blue No. 15 (WR 18043) in all treated groups (94% after intravenous dosing, 102% after low oral dosing, 95% after high oral dosing and 2% after dermal dosing). This suggests that when absorbed, that biliary excretion of HC Blue No. 15 (WR 18043) is important.
Urinary excretion was less important (1.9% after intravenous dosing, 1.2% after low oral dosing. 0.6% after high oral dosing and 0.06% after dermal application. The rate of urinary

excretion was similar for the intravenous and oral groups with the bulk of radioactivity excreted during the first 48h. In the dermal group. the rate of urinary excretion was very slow and the amount excreted was similar over each time interval.
By the end of the study, the average total remaining radioactivity in carcass and tissues was between 0.7 and 3% of the administered dose in all intravenous and oral groups, indicating no major accumulation of radioactivity after 96 hours. However, in some tissues, abdominal fat, adrenals, liver and kidney, the residual concentrations of HC Blue No. 15 (WR 18043) equivalents were much higher than the concentrations observed in blood or carcass. The relative high levels in kidney and liver reflect the extensive metabolism and excretion of the test substance.
In the dermal group. the highest concentration of HC Blue No. 15 (WR 18043) equivalents was observed in the treated skin, 2.03 mg/kg on average, or 0.5% of the administered dose. In addition. concentration in the liver was also higher than in other tissues or blood.
The average total recovery of radioactivity in the ADME groups was between 98% and 105%.
Four potential metabolite peaks of HC Blue No. 15 (WR 18043) were detected in faeces extracts and urine samples. It seemed that these were the result of hydroxylation, carboxylation and/or reduction by phase I metabolic enzymes. Hydroxylation was the most important. No parent compound was detected in the faeces.

Conclusion
Dermal penetration of ^{14}C_HC Blue No. 15 (WR 18043) was very low. ^{14}C_HC Blue No. 15 (WR 18043) administered orally was moderately absorbed. The absorbed HC Blue No. 15 (WR 18043) was metabolized in the liver, with excretion via bile into faeces the main route, with slower elimination after dermal application. No parent compound was found in the faecal extracts. Four potential metabolite peaks of HC Blue No. 15 (WR 18043) were detected. Hydroxylation, carboxylation and/or reduction by phase I metabolic enzymes occurred.

Ref.: 25

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

HC Blue n° 15

oxidative conditions

Absorption through the skin	**A**	**=**	**1.76 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**1.02 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose	**SAS x A x 0.001/60**	**=**	**0.02 mg/kg bw/d**
No Observed Adverse Effect Level (90 day study, oral route, rat)	**NOAEL**	**=**	**4 mg/kg bw/d**
35% bioavailability		**=**	**1.4**
MOS		**=**	**82**

3.3.14. Discussion

Physico-chemical properties
For the toxicological tests, a batch of the test substance had been used that is sufficiently characterized with respect to identity, purity and stability.
Data about homogeneity of the test substance or its formulations have not been supplied.

Irritation, sensitisation
Neat HC Blue n° 15 is irritant to mucous membranes. However, at a concentration of 2% (in water) slight irritation to mucous membranes was observed. The concentration of HC Blue n° 15 in the final product is 0.2 %.
Neat HC Blue n° 15 has irritant potential on rabbit skin.
In a LLNA test the test substance was not a skin sensitizer under the defined experimental conditions. However, the highest test concentration was too low. Therefore, sensitising potential has not been excluded.

Dermal absorption
The single experiment available did not conform to guidelines. 100mg/cm² of formulation was applied. Too few chambers were used (5) and the number of donors is not stated. The purity of HC Blue n° 15 is not given. The membrane thickness was inappropriate.
In view of the above, and as penetration of HC Blue n° 15 is low, the amount considered as being absorbed from an oxidative hair dye formulation containing 1.67% HC Blue n° 15 is considered as (mean +2SD) 1.76 µg/cm².

General toxicity
The no observed adverse effect level (NOAEL) for HC Blue No. 15 was considered to be 4 mg/kg bw/day in the sub-chronic (90 days) toxicity; 3 mg/kg bw/day for the dams and 30 mg/kg bw/day for foetuses in the prenatal development toxicity study.
The toxicokinetic/ADME study suggested moderate oral absorption and low dermal penetration of 14C_HC Blue No. 15. After being metabolized, excretion was mainly faecal.

No parent compound was found in faeces. From this study an oral bioavailability of 35% was derived.
No further reproductive toxicity studies have been provided.

Mutagenicity
Overall, the genotoxicity of HC Blue n° 15 is sufficiently investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Under in vitro conditions HC Blue n° 15 did not induce gene mutation neither in bacteria nor in mammalian cells. As an in vitro micronucleus test was negative, a clastogenic or aneugenic effect of HC Blue n° 15 was not found either.
The latter negative *in vitro* findings were confirmed in a well performed in vivo micronucleus test. HC Blue n° 15 exposure of mice did not result in an increase in cells with micronuclei.
Consequently, based on the present reports, HC Blue n° 15 can be considered to have no in vivo genotoxic potential and additional tests are not necessary.

Carcinogenicity
No data were provided

4. CONCLUSION

Based on the low Margin of Safety using on a worst case assumption of dermal absorption, HC Blue n° 15 cannot be considered safe for use in oxidative hair dye formulation at a maximum on-head concentration of 0.2%.

To come to a final conclusion, the SCCS is of the opinion that a state of the art percutaneous absorption study would be required.

5. MINORITY OPINION

Not applicable

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