Scientific Committee on Consumer Safety

SCCS

OPINION ON

Pigment Red 57

COLIPA n° C181

The SCCS adopted this opinion by written procedure on 12 October 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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ISSN 1831-4767 ISBN 978-92-79-30779-9
Doi:10.2772/9088 ND-AQ-12-029-EN-N

http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

ACKNOWLEDGMENTS

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Keywords: SCCS, scientific opinion, hair dye, Pigment Red 57, C181, directive 76/768/ECC, CAS 5858-81-1, EC 227-497-9

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Pigment Red 57, 12 October 2012

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 Pigment Red 57, with the chemical name Disodium 3-hydroxy-4-[(E)-(4-methyl-2-sulfonatophenyl)diazenyl]-2-naphthoate, was submitted in September 2003 by COLIPA[1].

The Scientific Committee on Consumer Products and Non Food Products intended for Consumers (SCCNFP) adopted at the 28th plenary meeting of 25 May 2004 the opinion (SCCP/0795/04) with the conclusion, that "the information submitted is inadequate to assess the safe use of Pigment Red 57. Before any further consideration, the following information is required:

* complete physico-chemical data;
* a percutaneous absorption study in accordance with the SCCNFP Notes of Guidance".

The current submission, submitted by COLIPA in July 2005, is an update of the submission I for Pigment Red 57. Pigment Red 57 is identical with CI 15850 also used as a colouring agent allowed for use in all cosmetic products. Pigment Red 57 is used as a direct dye in semi-permanent hair dye formulations at a maximum concentration on the scalp at 0.4%.

The current 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

Does the Scientific Committee on Consumer Safety (SCCS) consider Pigment Red 57 safe for consumers when used as a direct dye in non-oxidative hair dye formulations at a maximum concentration on the scalp at 0.4% taking into account the scientific data provided?

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

3. OPINION

3.1. Chemical and Physical Specifications

Pigment Red 57 is listed as CI 15850 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 1: colouring agents allowed in all cosmetic products.

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Pigment Red 57 (INCI name)

3.1.1.2. Chemical names

Disodium 3-hydroxy-4-[(E)-(4-methyl-2-sulfonatophenyl)diazenyl]-2-naphthoate
2-Naphthalenecarboxylic acid, 3-hydroxy-4-[(4-methyl-2-sulfophenyl) azo]-, disodium salt
3-Hydroxy-4-((4-methyl-2-sulfophenyl)azo)-2-naphthalenecarboxylic acid-disodium salt

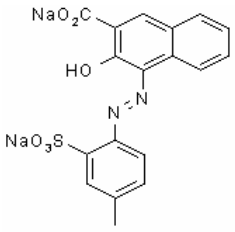
3.1.1.3. Trade names and abbreviations

Rouge Covanor W 3604 (LCW)
Lithol Rubin B
Lithol Rubin BK (calcium salt)
Japan Red 201
D&C Red Nº 6
COLIPA n° C181 (sodium salt)
CI 15850 (sodium and calcium salt + barium, strontium and zirconium lakes)

3.1.1.4. CAS / EC number

CAS: 5858-81-1
EC: 227-497-9

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{18}H_{14}N_2O_6S$. 2Na

3.1.2. Physical form

Orange-red powder

3.1.3. Molecular weight

Molecular weight: 430.35 g/mol

3.1.4. Purity, composition and substance codes

Chemical characterisation was performed by NMR

Comparison of batches

Batch	B 2095	Lot 7CO2
NMR content, % (w/w)	96.5	95.9
HPLC purity, % peak area 254 nm 500 nm	 98.7 99.6	 99.0 99.7
2-Amino-5-methyl-benzolsulfonic acid content, (% w/w)	<0.2	<0.15
3-Hydroxy-2-naphthalenecarboxylic acid content, % (w/w)	<0.4	0.4
3-Hydroxy-4-(4-Methylphenylazo)-2-naphthalenecarboxylic acid content, % (w/w)	<0.5	<0.5
p-Toluidine content, ppm	<15	<15
Metal content (ppm) Lead Arsenic Mercury	 10 3 1	 0.05 <0.05 <0.1
Sum of volatile matter, and chlorides and sulfates (calculated as sodium salts)	1%	Chloride <300 ppm Sulfate 340 ppm
Loss on drying, % (w/w)	0.4	0.5
Water content, % (w/w)	0.56	0.56
Sulfate ash, % (w/w)	33.4	35.7

3.1.5. Impurities / accompanying contaminants

See point 3.1.4. Purity, composition and substance codes

3.1.6. Solubility

Water: 3.78 g/L (20C, pH 7.94) determined by EC Method A.6 (ref. 3)

Acetone/water (1:1): 0.38%
DMSO: 0.5%
Ethanol/water (4:6): <0.3%

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 1.36 Determined by EC Method A.8 (ref. 1)

3.1.8. Additional physical and chemical specifications

Melting point: decomposition at 320 °C
Boiling point: /
Flash point: /
Vapour pressure: /
Density: 1.622
Viscosity: /
pKa: /

Refractive index: /
UV_Vis spectrum (200-800 nm): /

3.1.9. Homogeneity and Stability

Stable in a cosmetic formulation for 7 months at 25ºC (recovery: 95%).
In a certificate of analysis of Pigment Red 57 (Ref. 11), it is described that the stability of Pigment Red 57 in water (0.5% w/v, solution prepared by ultrasonication for 20 min) was tested by HPLC at 500 nm over a total period of 7days. During the test, the solution was stored at room temperature in the absence of light. The recovery under the storage conditions was 97%.

The solubility of Pigment Red 57 in the receptor fluid was 0.192 mg/ml; however, the stability testing was performed in 1 mg/ml solution (Ref. 20).

Comment
No supporting data was provided for the stability of Pigment Red 57 in the cosmetic formulation.

General Comments to physico-chemical characterisation

- The data on the stability of Pigment Red 57 in the receptor fluid cannot be accepted, the content of Pigment Red 57 in the "solution" was higher than its solubility.
- Pigment Red 57 was described to be stable in a cosmetic formulation for 7 months (recovery 95%). However, no supporting data was provided.

3.2. Function and uses

Pigment Red 57 is used as a "semi-permanent" hair dye at a maximum concentration of 0.4% in the finished cosmetic formulation.

Pigment Red 57 is listed as CI 15850 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 1: colouring agents allowed in all cosmetic products.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCNFP/0795/04

Rat
LD50 > 10,800 mg/kg bw (Na-salt)
LD50 > 9,800 mg/kg bw (Ca-salt)
LD50 > 5,000 mg/kg bw (Ca-salt)

Dog
LD50 > 9,800 mg/kg bw (Ca-salt)

Based on the observed high LD50 values of ≥ 5000 mg/kg bw, both salts and consequently *Pigment Red 57* are evaluated to be of low acute oral toxicity.

Ref.: 12, 13, 14

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/0795/04

Guidelines:	/
Species/strain:	New Zealand albino rabbit
Group size:	3 animals (sex not indicated)
Test item:	D&C Red 6, 10% dilution in propylene glycol
Batch:	/
Dosages:	1 ml
GLP:	/

D&C Red 6 was investigated as a 10% dilution in propylene glycol for its irritation potential in three New Zealand albino rabbits. The diluted test item was spread repeatedly (once daily, 5 days a week for two weeks) to the inner surface of one ear of each animal. The untreated ear served as control. At the end of the treatment period, the animals were examined for signs of irritation.

Results
No indication was found that D&C Red 6 might cause skin irritation under the described test conditions.

Ref.: 16

Comment

This was a generic results summary from a large number of cosmetic ingredients. Full experimental information was not provided.

3.3.2.2. Mucous membrane irritation

Guidelines: /
Species/strain: Albino rabbit, strain no given
Group size: 6 or more animals (sex not indicated)
Test substance: D&C Red 6 (lake)
Batch: Sun Chemicals certificate U0952
Dosages: 20 mg (equal to 0.2 ml of a 10% aqueous solution, twice daily)
GLP: /

D&C Red 6 was investigated with regard to its eye irritating and staining properties. 0.2 ml of a 10% aqueous solution was repeatedly applied to the conjunctival sac of one eye of each of the 6 or more animals per group for 4 weeks (twice daily; five days a week; 40 applications in total). One hour after each application, the eyes were scored for irritation according to the Draize system and for evidence of staining. In addition, scoring took also place the next day just prior to the first application of that day.

Results
A 10 % aqueous solution of D&C Red 6 did not cause any staining of orbital tissues under the described test conditions.
One hour after the application (figures given for day 5), an irritation score of 2 was revealed, indicating mild effects immediately after application. 24 hours after all applications of the 10% solution, no indications for eye irritation were noted.

Ref.: 17

Comment
This was a generic results summary from a large number of colour ingredients. Full experimental information was not provided.
Under the conditions of the experiment, D&C Red 6 caused transient irritation to the rabbit eye.

Assessment of the eye irritation potential in the Hen's egg test on the chorioallantoic membrane (HET-CAM)

Guidelines: /
Biological material: freshly fertilised eggs
Group size: 6 eggs per group
Test substance: Pigment Red 57
Batch: lot 7 CO2
Dosages: 1% aqueous dilution
GLP: in compliance

A HET-CAM assay was performed with a 1% aqueous dilution of Pigment Red 57. The diluted test item was applied onto the CAM of fertilised chicken eggs at day 9 of incubation and irritation parameters such as haemorrhage, lysis of blood vessels and protein coagulation were evaluated.

Results
The endpoint assessment as recommended for non-transparent test items was used. For this assessment, the test item was rinsed off 30 sec after application onto the CAM and evaluation of the parameters mentioned above was performed.

The 1% aqueous solution did not cause any damaging effect on the CAM as the obtained evaluation resulted in score 0. The test item has to be classified as a slight irritant at the test concentration of 1% in water.

Ref.: 18

Comment on status of HET-CAM
The HET-CAM (Hen's Egg Test-Chorio Allantoic Membrane) provides only supportive evidence for cosmetic ingredient safety assessment. This method can be recommended for use as screening tests for the identification of ocular corrosives and severe irritants, the protocol and decision criteria for the identification of ocular corrosives and severe irritants need to be optimized and undergo further validation (SCCS/1294/10).

3.3.3. Skin sensitisation

Taken from SCCNFP/0795/04

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429 (2000)
Species/strain:	Mice CBA/J
Group size:	5 females per group
Test substance:	D&C Red 6; CI 15850
Batch:	B2095 (LCW)
Concentrations:	0.5, 1, 2, 4% (w/v) in DMSO and water/acetone (1:1) mixed with olive oil (4:1)
GLP:	in compliance
Date:	2002

The skin sensitising potential of D&C Red 6 was investigated in CBA/J mice by measuring the cell proliferation in the draining lymph nodes after topical application onto the ears. 25 µl of 0 (vehicle only), 0.5, 1, 2 and 4% (exceeding the maximal solubility for both vehicles used) of D&C Red 6 in either DMSO or a mixture of water/acetone (1:1) with olive oil (4:1) were applied for three consecutive days to the surface of the ear of 5 female CBA/J mice per group. After application, the ears were dried for about 5 minutes by means of a hair dryer. A positive control (p-phenylenediamine at 1% in DMSO) was investigated in parallel under identical tests conditions.

At day 5 the mice received an intravenous injection of 250 µl phosphate buffered saline containing 21.4 µCi of [H3] methyl thymidine. About five hours later the mice were sacrificed by CO2-inhalation and the draining auricular lymph node was removed and weighed. After preparing a single cell suspension from the lymph nodes of each mouse, cells were precipitated by TCA and the radioactivity due to incorporation of [H3] methyl thymidine in the pellets was determined by liquid scintillation counting as disintegration per minute (dpm).
The mean dpm per treated group was determined and the stimulation index (test item compared to the concurrent vehicle control) was calculated.

Results
The weight of lymph nodes did not increase compared to the vehicle controls. The mean stimulation indices were not affected up to the highest concentration (above the maximum solubility) tested.

With the test item in DMSO mean stimulation indices of 1.2, 1.1, 1.0 and 1.2 were obtained for the 4 test concentrations of 0.5, 1, 2 and 4%, respectively.

In the second vehicle (water/acetone/olive oil) the indices were 1.0, 0.8, 1.2 and 1.6 for the 4 test concentrations.

As no relevant increase in the mean stimulation indices was observed, no indication was found that D&C Red 6 might be a skin sensitiser under the given test conditions.
The positive control (PPD, 1% in DMSO) caused an increase in the stimulation index by a factor of 7.8 and an increase of the mean lymph nodes weight by a factor of 1.8 and demonstrated the sensitivity and validity of the system used.

Conclusion
In the local lymph node assay, D&C Red 6 did not reveal any potential to be a skin sensitiser if tested with two different vehicles up to 4% (exceeding the maximum solubility). Based on these findings D&C Red 6 is evaluated not to be a skin-sensitiser.

Ref.: 19

3.3.4. Dermal / percutaneous absorption

New study, updated submission I, 2005

Percutaneous Absorption through pig skin *in vitro*

Guideline:	OECD 428 (2004)
Tissue:	Split thickness pig skin samples from back and flanks (113 µm thick).
Group size:	6 samples from 3 pigs (1 male, 2 females); 1 served as vehicle control.
Skin integrity:	tritiated water
Diffusion cell:	6 permeation chambers (Teflon-chambers with 9.1 cm^2 surface, in-house development)
Test substance:	WR21176
Batch:	7CO2
Purity:	99.0 area% (HPLC at 254 nm); dye content 96.0%
Test item:	Color cream formulation with 0.4% dye (WR21176), batch VDE/0020/1
Dose volume:	400 mg (100 mg/cm^2) containing 0.4% WR21176
Receptor fluid:	phosphate buffered saline
Solubility receptor fluid:	0.192 mg/ml (at pH 7.3)
Stability receptor fluid:	85% recovery after 3 days of a 1 mg/ml solution 83% recovery after 7 days of a 1 mg/ml solution
Method of Analysis:	HPLC, detection and measurement at 492nm
GLP:	in compliance
Study period:	19 – 26 September 2005

The percutaneous absorption of 0.4% WR21176 in a typical direct hair dye formulation was studied through pig skin. HPLC was used for detection with measurements in the receptor fluid being made at 16, 24, 40, 48, 64 and 72 hours after application. At the end of the experiment, the skin membrane was heat treated and the upper skin mechanically separated.

The limit of detection of WR21176 by HPLC was taken as 3.6ng/injection for calculations of mean, corresponding to 9ng/cm^2 for skin and 10.8ng/ml for receptor fluid.

Results

72 hour absorption of 0.4% WR21176 from a typical direct hair dye formulation

	Skin	Integrity-Test	1)		2)		3)		4)		1) + 2) + 3) + 4)	
	No	^3H_2O Permeation (4 hours cumulative)	Receptor fluid (72 hours cumulative)		Lower skin (72 hours cumulative)		Upper skin (72 hours cumulative)		Rinsing solution (after 60 minutes)		Total****	
		[% Dose]	[µg/cm²]	[% Dose]	[µg/cm²]	[% Dose]	[µg/cm²]	[% Dose]	[µg/cm²]	[% Dose]	[µg/cm²]	[% Dose]
Application of 0.4 mg of WR21176 in 100 mg of vehicle* per 1 cm² of skin	2	1.2	BLD** (0.011)	BLD** (0.003)	BLD** (0.009)	BLD** (0.002)	BLD** (0.009)	BLD** (0.002)	374.62	93.66	374.65	93.66
	4	1.1	BLD** (0.011)	BLD** (0.003)	BLD** (0.009)	BLD** (0.002)	BLD** (0.009)	BLD** (0.002)	383.34	95.84	383.37	95.84
	6	1.0	BLD** (0.011)	BLD** (0.003)	BLD** (0.009)	BLD** (0.002)	BLD** (0.009)	BLD** (0.002)	393.67	98.42	393.70	98.43
	8	1.4	BLD** (0.011)	BLD** (0.003)	BLD** (0.009)	BLD** (0.002)	BLD** (0.009)	BLD** (0.002)	403.82	100.96	403.85	100.96
	12***	2.2	BLD** (0.011)	BLD** (0.003)	BLD** (0.009)	BLD** (0.002)	BLD** (0.009)	BLD** (0.002)	385.31	96.33	385.34	96.34
Control skin (vehicle only)	10	2.6	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
Mean		**1.6**	**BLD** (0.011)**	**BLD** (0.003)**	**BLD** (0.009)**	**BLD** (0.002)**	**BLD** (0.009)**	**BLD** (0.002)**	**388.86**	**97.22**	**388.89**	**97.22**
± S.D		0.7	-	-	-	-	-	-	12.65	3.16	12.65	3.16
(n)		(6)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)

**BLD, below level of detection.
*** Outlier, not considered for calculation of mean.
**** Total considered with respect to assumption that for each limit of detection (LOD), the amount of LOD for the fraction is taken into account for the calculation.

Only 4 chambers were considered available (3 donors) for calculating absorption. However, WR21176 was below the LOD in all samples. Therefore, based on the LOD a maximum absorption of 0.029 µg/cm² (0.007% of the applied dose) of WR21176 from a direct hair dye containing 0.4% of WR21176 was determined (receptor fluid + lower skin + upper skin).

Ref.: 20

Comment
Too few chambers were available. The dose was too high.
With the methodology used, any WR21176 present in the receptor or skin was below the limit of detection. Calculation of biological availability (amount considered as absorbed) was, therefore, based on summations of LOD in the compartments.
The solubility in the receptor fluid was 0.192 mg/ml; however, the stability testing was performed in 1 mg/ml solutions.

Despite the limitations of this study, which would ordinarily have excluded the results from further consideration, use of the limits of detection of WR21176 provides an acceptable estimation of potential absorption.

Taken from SCCNFP/0795/04

Percutaneous Absorption *in vitro*

Guideline: /
Tissue: Porcine ear skin obtained by dissection
Method: Flow-through Franz diffusion cells
Test substance: Pigment Red 57 tested in acetone/water (1:1) solution (2-3 mg /200 µl) and in a commercial formulation (0.5%) (1 mg of the pure dye in the formulation)
Batch: B2095
Purity: 95%

Dose level:	About 2-3 mg /cm² of the dye in the acetone/water solution (1:1) and about 1 mg/cm² of the dye in the formulation (200 mg formulation /cm²)
Receptor fluid:	0.9% Na Cl solution, pH 3.0
Replicate cells:	6 cells
Analytical method:	HPLC (Detection at 508 nm); Quantitation limit: 0.021 µg/ml
GLP:	In compliance

The skin penetration of Pigment Red 57 was evaluated in a flow-through Franz diffusion cell system using porcine ear skin (thickness: 300-400 µm). The integrity of the skin was checked by conductivity and no loss of barrier properties of the skin was detected. The solubility of the dye in the receptor fluid was not provided.
The dye formulations were applied on the skin surface (exposure area: 1cm²) for 30 min. Then, the skin surface excess was removed by washing the skin three times with 1 ml of a 10% diluted shampoo formulation. For the remaining exposure time of the experiments (24 hours), the donor chamber was filled with 1 ml saline solution and covered with Parafilm. Fractions of the receptor fluid were collected at different times between 0 and 24 hours. At the end of the experiment, the epidermal membrane was mechanically separated from full thickness skin. The isolated skin piece was separated into the "upper skin" (SC + upper stratum germinativum) and the "lower skin" (lower stratum germinativum + upper dermis). Both skin compartments were extracted separately (tetrabutylammonium hydrogen sulphate- ammonium acetate buffer and acetonitrile at pH 2.0) and their dye content quantified by HPLC.

Results
Under the present experimental conditions, total recoveries of 109.7% ± 15.0 and 111% ± 4.2 were obtained for the dye incorporated in an acetone/water (1:1) solution and in a formulation, respectively. Most of the hair dye applied on the skin surface was removed with the washing procedure (> 99%). The content of the dye in the receptor fluid was 0.61 and 0.60µg/cm² for the dye tested in acetone/water or as a part of a formulation, respectively.
Global percutaneous absorption values are reported without considering the separated skin compartments. A skin penetration rate for Pigment Red 57 of 1.01 µg/cm² (0.039% of the applied dose) is reported if the test substance is applied in acetone/water whereas a skin penetration rate of 0.94 µg/cm² (0.091% of the applied dose) is reported for the formulation.

Ref.: 9 (subm I)

Comments
- The solubility of the dye in the receptor fluid is not provided.
- The intervals of the recoveries are very high.
- The pH of the receptor fluid (3.0) is not appropriate.
- The procedure used for the isolation of the "upper and lower" skin is not clear.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (30 days) oral toxicity

No data submitted

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

Taken from SCCNFP/0795/04

Data for the calcium-salt

30-day oral toxicity study in rats

Administration of 1 g/kg bw/day of the dye by stomach tube, 5 days/week over 30 days (a total of 22 doses), to groups of 20 males and 20 females slightly reduced growth (although food consumption was unaffected), whilst kidney weight was increased and kidney damage was evident on microscopic examination. All these effects were reversible over a two week recovery period. There were no effects on the blood and urine, or on the weight and microscopic appearance of the liver, adrenals and spleen. In view of the red discoloration of faeces (but not the urine), the author of the study considered it unlikely that Lithol Rubine BK had been absorbed through the gastrointestinal tract.

Ref.: 14

18-week feeding toxicity study in rats

According to a short abstract, groups of five male and five female rats fed diets containing 0.25, 0.5, 1 or 2% Lithol Rubine BK (approximately 125, 250, 500 or 1000 mg/kg bw/day, respectively) for 18 weeks, showed no effects on food intake, body weight, blood composition, organ weights (details not given) or the appearance of (unspecified) tissues on microscopic examination.

Ref.: 14

13-week feeding study in dogs

In a dog study, administering doses of 0.5 % (week 1 and 2), 1.0% (week 2 and 3), 1.5% (week 5-10) and 2.0% (week 11-13) to one animal per sex, no substance related findings were observed. The only effects noted were diarrhoea (observed a few days before dose was increased to 1.5%) and vomiting (observed a few days after increase to 2.0%).

Ref.: 15

18-month dermal toxicity study in mice

In a mouse study (limited reporting), a 1 % aqueous suspension of Lithol Rubine BK (about 50 mg/kg bw/application] was applied to 50 males and 50 females twice weekly for 18 months.
Survival was unaffected and there were no clear effects on the gross or microscopic appearance of a range of tissues.

Remark
The above mentioned limited study is not suitable to derive a reliable reference figure for the final risk assessment. Nevertheless, the lack of any effects at the highest tested dose level of 50 mg/kg bw allows some conclusions with regard to the expected effects after repeated dermal applications.

Ref.: 15

Taken from EFSA Scientific Opinion on the re-evaluation of Litholrubine BK (E 180) as a food additive:

An OECD 422 study not included in the applicant dossier has been recently evaluated by the EFSA panel on food additives and nutrient sources added to Food. This study was done in rats for an exposure period of 42 days in males and 17 days in females, in accordance with OECD Test Guideline 422 and GLP conditions. Decreased general biochemistry parameters in serum and Glutamic Oxaloacetic acid Transaminase (GOT) levels as well as increased kidney weights and histopathological lesions in renal tubular epithelium were observed in males receiving 1000 mg/kg bw/day Litholrubine BK (OECD SIDS, 1994). No effects were reported on reproductive/developmental toxicity parameters measured in this study. Female rats that received 100 or 1000 mg/kg bw/day showed statistically significant decreases in thymus weights in comparison to the controls. Decreases in thymus weight were not statistically significant in females of the mid-dose group. Relative thymus weight was

statistically significantly decreased in females of the low-dose group only. No further statistically significant differences in organ weights were observed in males or females.
Histopathological examinations showed alterations predominantly occurring in the kidney. The lesions included regenerated renal tubular epithelium in male rats receiving 300 mg/kg bw/day or higher. In all treated female groups, the incidence of foamy tubular epithelial cells was increased compared to controls. Although the incidence was similar in all dose groups, the severity of this lesion was slightly increased in the high-dose group only. In addition, increased incidences of necrotic tubular epithelium were seen in all treated groups compared to controls, however no dose-effect relationship either in incidence or severity was observed. The Panel, having consulted the tables in the original Japanese and the English abstract of this study, concludes that there was a NOAEL of 100 mg/kg bw/day for males and that the NOAEL for females was below 100 mg/kg bw/day (the lowest dose tested). Based on the lack of a clear dose-response relationship in this study the Panel was unable to identify a suitable NOAEL, LOAEL or Bench Mark Dose (BMD) to establish an ADI and concludes that the existing SCF ADI of 0-1.5 mg/kg bw/day should be withdrawn.

Ref.: Add ref A, B

3.3.5.3. Chronic (> 12 months) toxicity

Taken from SCCNFP/0795/04

Data for the calcium-salt

2-year feeding study in dogs
Dietary levels of 0.015, 0.1 and 1.0 % (about 250 mg/kg bw/day) of the test substance were fed to dogs (3 animals per sex) for 2 years. A concurrent control group of 6 animals per sex was also investigated. General conditions, body weight, food consumption, survival rate, blood chemistry, clinical chemistry, urinalysis were investigated. At necropsy, organ weights were determined and macroscopic and microscopic investigations were performed. According to the available summary, no substance related effects were noted and a dose of 1.0 % was determined as the no effect level. The slight increased thyroid weights were not considered as a pathological effect.
From information given in the toxicity profile provided by BIBRA, it seems that this conclusion was drawn based on the fact that no correlating effects were seen at macroscopic and microscopic investigation of this tissue.

Ref.: 14

Data for the sodium-salt

Chronic oral toxicity study in rats (F1 generation)
Dietary administration for up to 2 years of 0.05, 0.3 or 2% of Pigment Red 57 [roughly 25, 150 or 1000 mg/kg bw/day] to groups of 70 males and 70 females (the offspring of rats which had been treated at the same levels for 60 days prior to mating and throughout pregnancy and lactation), resulted in a dose-related reduction in growth, particularly in the male rats. In addition, increased mortality was observed in the male rats at the top dose. Blood composition was unaffected and, apart from the colour of the urine, no treatment-related effects were noted on urinary analysis. At the end of the study, the weights and gross appearance of the major organs were unaffected, except in the high-dose male rats which showed organ weight variations relative to their reduced body weight. Microscopic examination of the major tissues (limited to the control and high-dose animals) revealed an increased incidence of kidney changes in both males and females. When pathologists from the US Food and Drug Administration subsequently examined tissue sections from the kidneys of all treated rats, they concluded that Lithol Rubine B exacerbated a spontaneous kidney disease of aged rats (chronic progressive nephrosis) in the mid- and high-dose males and in the high-dose females. An acceleration of testicular changes (degeneration of the testicular tubules), common in ageing rats, was also reported in high-dose males, but the increased incidence was of no statistical significance. The NOAEL was 150 mg/kg bw/day.

Ref.: 13

Chronic oral toxicity study in mice
Groups of 60 males and 60 females that were exposed to concentrations of 0.05, 1 or 5% Pigment Red 57 in their diet (approximately 75, 1500 or 7500 mg/kg bw/day, respectively) for 2 years, showed no changes in food consumption, body weight gain, or blood composition. At the end of the study, the weights of the brain, kidneys, liver and spleen were unaffected and there were no abnormalities evident on gross examination. The treated males showed increased mortality, being statistically significant in the top-dose group, and microscopic examination (limited to the control and high-dose groups only) revealed degenerative kidney changes in the treated males.

Ref.: 13

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Taken from SCCNFP/0795/04

Bacterial Reverse Mutation Assay

Guideline:	OECD 471 (July 1997)
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, TA 1537
Replicate:	triplicates in 2 independent experiments
Test substance:	D&C RED NO.6 W 003
Solvent:	DMSO
Batch:	B2095 (LCW)
Purity:	98.7 area % (HPLC), 96.5 weight % (NMR)
Concentrations:	experiment 1: 0, 1, 10, 100, 1000 and 5000 µg/plate without and with S9-mix experiment 2: 0, 30, 100, 300, 1000 and 3000 µg/plate without and with S9-mix experiment 2 TA102 only: 0, 1, 3, 10, 30, 100 and 300 µg/plate with S9-mix experiment 3 TA102 only: 0, 0.3, 1, 2, 3, 5, 10 and 20 µg/plate with S9-mix
Treatment:	direct plate incorporation with 48 h incubation
GLP:	in compliance
Study period:	13 March 2002 – 11 April 2002

D&C RED NO.6 W 003 was investigated for the induction of gene mutations in strains of *S. typhimurium*. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system (purchased; not checked). The experiments were performed according to the direct plate-incorporation test. Toxicity was evaluated on the basis of a reduction in the number of spontaneous revertant colonies. Negative and positive controls were in accordance with the OECD guideline.

Results
Precipitation was observed at concentrations of 3000 µg/plate and above. Toxicity is not reported for treated cultures. For controls toxicity was within the normal historical range. A biological relevant increase in the number of revertants was not observed. Exclusively, in TA102 in the presence of S9-mix a small increase in the number of revertants was observed. As these effects did not show a dose response relationship and did not fulfil the criteria for a positive result, they were considered of no biological significance.

Conclusion

Under the test conditions used, it is concluded that D&C RED NO.6 W 003 is not mutagenic in this gene mutation test in bacteria.

Ref.: 21

Bacterial Reverse Mutation Assay

Guideline:	OECD 471 (1997)
Species/strain:	*Salmonella typhimurium* TA98; TA100; TA102; TA1535; TA1537
Replicate:	triplicates in 2 independent experiments
Test substance:	Pigment Red 57 (WR21176)
Solvent:	DMSO
Batch:	7 CO2 (Fa.Toshiki Japan)
Purity:	99.0% (area%)
Concentrations:	experiment I: 3, 10, 33, 100, 333, 1000, 2500, 5000 µg/plate without and with S9-mix experiment II: 156.25, 312.5, 625, 1250, 2500, 5000 µg/plate without and with S9-mix
Treatment:	pre-incubation method with 30 minutes pre-incubation and at least 48 h incubation, without and with S9-mix.
Positive controls:	Congo red in addition to guidelines
GLP:	in compliance
Study period:	28 October 2005 – 13 December 2005

Pigment Red 57 was investigated for the induction of gene mutations in strains of *Salmonella typhimurium* (Ames test). The study was performed according to the pre-incubation assay, with and without hamster liver S9-mix, obtained from non-induced Syrian golden hamsters, to investigate the potential of the azo dye to induce gene mutations in bacteria. The test item was suspended in DMSO. Test concentrations were based on the results of a pre-test with all strains and 8 test concentrations up to the prescribed maximum concentration of 5000 µg/plate measuring toxicity and mutagenicity. Toxicity was measured as a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn. This pre-experiment is reported as experiment I since evaluable plates at 5 concentrations were available for all strains used. Appropriate negative (DMSO) and positive controls were included in the assay. Congo red was included as positive control to demonstrate the activity of the hamster liver S9-mix to cleave the azo bond.

Results
Normal background growth was observed at all concentrations tested and there was no reduction in revertants in the first experiment. In the second experiment a toxic effect was observed, evident as a reduction in revertant colonies, in TA102 without S9-mix at the highest concentration tested and in TA98 with S9-mix from 1250 µg/plate and upwards. There was no biological relevant or statistical significant increase in revertants at any concentration tested either with or without metabolic activation. All positive controls including Congo red induced a distinct increase in mutant frequency.

Conclusion
Under the experimental conditions used Pigment Red 57 was not mutagenic in this gene mutation test in bacteria both in the absence and the presence of S9 metabolic activation.

Ref.:24

Taken from SCCNFP/0795/04

***In Vitro* Mammalian Cell Gene Mutation test**

Guideline: OECD 476 (1997)

Species/strain:	Mouse Lymphoma cell line L5178Y
Replicates:	duplicate cultures in 2 independent experiments
Test substance:	RED 201 WR 21176
Batch:	7 CO2 (Fa. Toshiki Japan)
Purity:	95.9 weight % (NMR spectrum)
Vehicle:	culture medium RPMI 1640
Concentrations:	10, 20, 40, 80, 160 and 320 µg/ml, without and with S9-mix.
Treatment	experiment 1: 4 h both without and with S9-mix; expression period 72 h and a selection period of 10-15 days.
	experiment 2: 24 h without S9-mix; expression period 48 h and a selection period of 10-15 days.
GLP:	in compliance
Study period:	5 February 2002 – 9 July 2002

RED 201 WR 21176 was assayed for mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of metabolic activation. In the pre-test liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats was used as exogenous metabolic activation system, in the main experiments liver S9 fraction from non-induced Syrian golden hamsters was used. Test concentrations were based on the results of a pre-test measuring cell density immediately after treatment and at each day of the growth period and relative suspension growth using concentrations up to 5000 µg/ml which is the prescribed maximum concentration according OECD.
In the main test, cells were treated for 4 h (experiment 1) or 24 h (experiment 2, without S9-mix only) followed by an expression period of 72 h and 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. An increased occurrence of small colonies indicated by a low large/small colonies ratio (<4) was associated with clastogenic effects and/or chromosomal aberrations. Toxicity was measured as percentage relative survival of the treated cultures relative to the percentage relative survival of the solvent control cultures. Negative and positive controls were in accordance with the OECD guideline.

Results
Based on the solubility of RED 201 WR 21176 and the results of the pre-test, 320 µg/ml was chosen as the top concentration both without and with S9-mix in both experiments.
In the main experiments, the appropriate level of toxicity (about 10-20% survival after the highest dose) was never reached. However, turbidity was observed at 80 and 160 µg/ml and heavy precipitation occurred at 320 µg/ml.
A biological relevant increase in the mutant frequency was not found following treatment with RED 201 WR 21176 at any dose level tested, either in the absence nor in the presence of S9-mix. A relevant and reproducible shift in the ratio between large and small colonies was not observed either.

Conclusion
Under the experimental conditions used, RED 201 WR 21176 was considered not mutagenic in this *tk* gene mutation assay in mouse lymphoma cells.

Ref.: 25

Comment
In the main experiments, the appropriate level of toxicity (about 10-20% survival after the highest dose) was never reached making the negative result less reliable.

Taken from SCCNFP/0795/04

***In vitro* Micronucleus Test**

Guideline:	Draft OECD 487	
Species/strain:	human lymphocytes from the pooled blood of 2 or 3 male donors	
Replicates:	duplicate cultures in two independent experiments	
Test item:	Pigment Red 57 (WR21176)	
Batch:	7 CO2	
Purity:	99.0%	
Vehicle:	purified water	
Concentrations:	experiment 1:	100, 150, 200 µg/ml without S9-mix 50, 75, 100 µg/ml with S9-mix:
	experiment 2	75, 150, 200 µg/ml without S9-mix 150, 200, 250 µg/ml with S9-mix
Treatment:	experiment 1:	24 h PHA stimulation, 20 h treatment without S9-mix, harvest time 48 h after the start of treatment 24 h PHA stimulation, 3 h treatment with S9-mix, harvest time 48 h after the start of treatment
	experiment 2:	48 h PHA stimulation, 20 h treatment without S9-mix, harvest time 48 h after the start of treatment 48 h PHA stimulation, 3 h treatment with S9-mix, harvest time 48 h after the start of treatment
GLP:	in compliance	
Study period:	26 September 2005 – 14 November 2005	

Pigment Red 57 was tested in the *in vitro* micronucleus assay for the induction of structural or numerical chromosome aberrations. The test substance was dissolved in purified water and the concentrations used in the main study were based on a preliminary cytotoxicity range finding study. The highest concentration evaluated was based on post treatment precipitate. The substance was tested both with and without S9-mix. Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Appropriate negative (water) and positive controls were included.

Results
Both in the absence and presence of metabolic activation, biologically relevant and statistically significant increases in the number of lymphocytes with micronuclei were not observed in both experiments at any of the concentrations of Pigment Red 57 tested with two exceptions. However, these positive findings fell within the range of the historical control data and were therefore considered as not biologically relevant.

Conclusion
Under the experimental conditions used Pigment Red 57 did not induce an increase in lymphocytes with micronuclei and, consequently, Pigment Red 57 is not genotoxic (clastogenic and/or aneugenic) in cultured human peripheral blood lymphocytes.

Ref.26

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

Taken from SCCNFP/0795/04

Mammalian Erythrocyte Micronucleus test

Guideline:	OECD 474 (1997)
Species/strain:	NMRI mice
Group sizes:	5 mice/sex/group
Test substance:	RED 201 WR 21176

Batch:	7 CO2 (Fa.Toshiki Japan)
Purity:	95.9 weight% (NMR spectrum)
Vehicle:	0.5% aqueous carboxymethylcellulose
Dose levels:	500, 1000, 2000 mg/kg bw for 24 hours
Treatment:	oral
Sacrifice times:	24 h or 48 h (high dose group only) after treatment.
GLP:	in compliance
Study period:	7 February 2002 – 2 August 2002

RED 201 WR 21176 has been investigated for induction of micronuclei in the bone marrow cells of mice. Dose selection was based on the result of a pre-experiment for toxicity in which 2 mice/sex were treated orally with 2000 mg/kg bw RED 201 WR 21176. The animals were examined for acute toxic symptoms at intervals of around 1, 2-4, 6, 24, 30 and 48 h after administration.
In the main experiment mice were exposed orally to 0, 500, 1000 and 2000 mg/kg bw. The animals of the highest dose group were examined for acute toxic symptoms at intervals around 1, 2-4, 6 and 24 h after treatment. Bone marrow cells were collected 24 h or 48 h (high 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 ratio). Bone marrow preparations were stained and examined microscopically for the NCE/TE ratio and micronuclei. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-experiment in which 2 mice/sex were treated with 2000 mg/kg bw RED 201 WR 21176, all mice showed reduction of spontaneous activity up to 6 h after dosing, ruffled fur up to 48 h after dosing and some mice showed eyelid closure. On the basis of these results 2000 mg/kg bw was chosen as the highest dose.
In the main experiment similar toxic signs and additionally abdominal position and apathy were observed. In males but not in females the PCE/TE ratio decreased after treatment indicating that bone marrow was exposed and that RED 201 WR 21176 was toxic to bone marrow cells. Bioavailability was also demonstrated by the toxic signs observed. In comparison to the concurrent vehicle controls there was no biologically relevant or statistically significant increase in the number of erythrocytes with micronuclei at any preparation interval and dose level.

Conclusion
Under the experimental conditions used, RED 201 WR 21176 did not induce an increase in the number of polychromatic erythrocytes with micronuclei in the bone marrow of the treated mice and, consequently, RED 201 WR 21176 is not genotoxic (clastogenic and/or aneugenic) in polychromatic erythrocytes of mice

Ref.: 27

3.3.7. Carcinogenicity

Taken from EFSA Journal 2010;8(5):1586

Groups of 60 male and 60 female Charles River CD1 mice were fed diets containing 0, 0.05, 1.0, or 5.0% D&C Red No. 6 (Litholrubine B) for 104 weeks (equivalent to 0 75, 1500 or 7500 mg/kg bw/day Litholrubine B); 2 groups of 60 animals of each sex were used as controls. Individual body weights were recorded weekly, biweekly and monthly. Haematological examinations were carried out on 10 mice/sex/group at 3, 6, 12 and 18 months. At termination of the study, all animals were necropsied and the weights of the brain, kidneys, liver, and spleen were recorded. Complete histopathological examination was carried out on the control and high-dose groups only. No statistically significant differences were observed in food consumption, body weight gain, or haematological parameters mentioned above except for a depressed reticulocyte count in the high-dose

groups relative to controls after 18 months, although the counts were within the expected range for mice of that age according to the report.

Beginning at week 64, there was a treatment-related increase in mortality in males, and survival was statistically significantly reduced in the 5% dose group at 91 and at 104 weeks; increased mortality was not observed in females. No toxicologically-significant dose-related differences in organ weights or gross morphology were observed. Histopathological examination of animals in the high-dose group revealed a variety of degenerative, inflammatory, proliferative, or neoplastic lesions which according to the report were commonly associated with aging mice, occurring with similar frequency or sporadic distribution in controls. Exceptions were degenerative renal changes, which occurred with higher incidence among treated males from the high-dose group, and alveolar adenomas, which were the most common tumours occurring in the study. Compared to controls, statistically significant increases in the unadjusted incidence of alveolar adenomas were seen in high-dose group males, but in the report these were considered of dubious toxicological significance because of unequal sampling of the low- and mid-dose groups and because of earlier diagnosis associated with the increased mortality in the high-dose group. No others statistically significant treatment-related increases were reported in tumours or non-neoplastic lesions at other sites, the occurrence of which were considered in the report to be incidental or common for aged mice (IRDC, 1981a).

The JECFA evaluation (1987) concluded that "in the long-term mouse study, there was a dose-related increase in mortality and renal pathology, but detailed histopathology was not conducted on the low and intermediate-dose groups.

Rats

In a chronic toxicity study with an in utero phase, D&C Red No. 6 (Litholrubine B) was administered to Charles River CD rats at dietary concentrations of 0, 0.05, 0.3, or 2% (equivalent to 0, 25, 150, 1000 mg/kg bw/day). In the *in utero* segment of the study, 60 rats of each sex were assigned to each treatment group and then mated after receiving the diet for 60 days. A minimum of 35 litters per dosage level was used to select 70 rats of each sex per group for the long-term segment of the study. In the long-term phase, the pups were weaned onto their respective diets at 21 days and maintained on these diets throughout the remainder of the experiment. Individual body weights and food consumption measurements were recorded. Ophthalmoscopic examinations, haematology, serum biochemical examinations and urinalysis were performed at regular time-points and after 24 months for females in the long-term phase. An interim sacrifice and necropsy of 10 rats/sex/group was conducted after 12 months of treatment. For animals killed at the interim or terminal sacrifices, brain, kidney, liver, spleen, testes, thyroid, heart, adrenals, uterus, and ovaries were weighed. Complete histopathological examination was carried out on the control and high-dose groups only.

In the long-term phase, mean food consumption values were similar for control and treated rats, but there was a treatment-related depression in body-weight gain, most marked in the high-dose group. Males showed a larger decrement in body-weight gain than females in the same dose group; the deficit compared to controls reaching about 19% for high-dose males by week 91 of the study. There was an accelerated mortality rate in male rats in the high-dose group and, for males, the study was terminated at week 95 when there were only 9 survivors in the high-dose group compared with 17 and 29 in the two control groups. No changes considered to be related to treatment were reported in the haematological and clinical biochemical examinations and, apart from the colour of the urine, no differences attributable to treatment were observed in urinalysis values. At 20 months, in an additional haematological investigation on 1 low-dose male and 1 high-dose male markedly elevated leucocyte counts were reported, which were attributed to a probable infection with Mycoplasma pulmonis.

No compound-related macroscopic changes were detected. There were no statistically-significant variations in mean organ weights at the 12-month interim sacrifice, and subsequent statistically-significant variations in the high-dose males were related in the report to the decrement in mean body weight. Histopathological examinations revealed a higher incidence of chronic nephritis, renal tubular epithelial hyperplasia, myocardial fibrosis, reticular hyperplasia, and pigment deposition in the spleen than in the controls. Compared to controls, a higher incidence of atrophy/degeneration of testicular tubules was reported in high-dose male rats that died during the study and from 12 months of treatment to termination. These changes were considered common in aging rats and no specific compound-related effect was identified other than an acceleration of these changes. There was no statistically significant increase in incidence of the above lesions at termination.

The number of malignant tumours in the males rats, was significantly increased (P>0.01) using the Kruskal-Wallis test for adjusted trend, but it was suggested that this single value was not toxicologically significant because of the small number of tumours present (unadjusted incidence was 4% in the controls and 9% in the high-dose group). There was an unusually high incidence of pituitary adenomas in one control group of males; the incidence in the high-dose group was not statistically significant increased over either control group. The unadjusted incidence of Leydig cell adenomas in males was reported to be 2% in controls and 6% in high-dose animals, not statistically significant at the 0.05 level, but tests for unadjusted trend and homogeneity of life table curves were statistically significant at the 0.01 level. However, they were of doubtful toxicological significance because of the small number of tumours involved (IRDC, 1981b).

The JECFA evaluation (1987) concluded that "The long-term study in rats was complicated by high mortality rates,which led to premature termination of the study for males. In addition, only limited histopathological examinations were conducted. In view of these limitations, it was not possible to determine an unequivocal no-effect level in either study.

Dermal study (calcium salt)

Mice
Tumour incidence was not increased in 50 male or 50 female mice given uncovered applications, twice weekly with 1% aqueous suspension of Pigment Red 57 (calcium salt) (about 50 mg/kg bw/application) for 18 months. Tissues from the skin were microscopically examined, as were other tissues that appeared abnormal on gross examination.

Ref.: 14

Comment
No information is given concerning the strain of mice used, the site(s) of application or other experimental details.

General comment on Carcinogenicity
Pigment Red 57 has been studied in long term carcinogenicity experiments after oral administration of mice and rats and skin application of mice. Due to the inadequacy of the studies, no conclusions concerning potential carcinogenic effects of Pigment Red 57 can be drawn from the experiments.

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Taken from SCCNFP/0795/04

Developmental toxicity in Rats
In a developmental toxicity study in rats, Lithol Rubine BK was administered at doses of 5, 16 or 50 mg/kg bw/day by stomach tube on days 6 - 15 of pregnancy to 20 females. No adverse effects on maternal weight gain, number of resorptions (early embryo/foetal deaths), foetal weight and viability, litter size, or the incidence of foetal malformations or skeletal aberrations were noted.

Ref.: 13

According to an unpublished report, Lithol Rubine B had no effect on fertility, pregnancy or lactation when administered to groups of 60 male and 60 female rats at dietary levels of 0.05, 0.3 or 2% (approximately 25, 150, or 1000 mg/kg bw/day) prior to mating and throughout pregnancy and lactation. When the offspring (70 males and 70 females) from each group was maintained on the respective parental diets for further 2 years, the high-dose males showed, from month 12 onwards, an acceleration of testicular changes (degeneration of the testicular tubules), a common effect in ageing rats. However, at the termination of the study, the increased incidence was not of statistical significance.

Ref.: 13

In a limited unpublished three-generation rat study, dietary administration of 0.5, 5, 15 or 50 mg/kg bw/day to groups of ten males and 20 females had no effect on maternal or foetal body weights, number of resorptions, or survival of the offspring. There was a reduction in fertility in the second generation at 50 mg/kg bw/day, but this was not seen in the third generation at any dose level (study cited in Ref. 3). As the effect was noted in the second but not in the third generation, this finding is considered as an incidental finding and not interpreted as an indication for a reproductive toxic effect of the test item.

Developmental toxicity in Rabbits
According to an unpublished study, groups of ten female rabbits given 5, 16 or 50 mg/kg bw/day by stomach tube on days 6 – 18 of pregnancy showed no adverse effects on maternal weight gain, number of resorptions, litter size, foetal weight and viability, or the incidence of foetal malformations.

Ref.: 14

Furthermore in reference 14, a study performed with mink is cited but without giving any details on study design, etc.

Results
None of the available studies for either the sodium or the calcium salt gave any indication that the azo-dye might be a reproductive toxin up to the highest dose tested.
Although none of the reported studies is in line with current guideline requirements and the available information is rather limited for all reported studies, including a 3-generation study and teratogenicity studies in 2 species support the overall conclusion, that Pigment Red 57 does not have to be considered as a reproductive toxic substance.

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

A 26-year-old Korean woman presented with a pruritic and irritating sensation, with dryness and slight swelling of the lips, of 7 days duration. A clinical examination showed erythematous papules and vesiculation with scaly fissures on and around the lower lip. She had a previous history of frequent cheilitis on using dark red-coloured lipsticks for 6 years.

She was patch tested with the Korean standard series and a cosmetics series and showed positive reactions to thimerosal and her lipstick. A further patch test with the 32 individual ingredients of the lipstick formulation provided by the manufacturer showed a ++ reaction to D&C Red no. 7 calcium lake (CI 15850) 20% aq.; the other ingredients were negative. The patient discontinued using this lipstick, and her symptoms improved.

Ref. C

Comment
This is an isolated case report. Presumably a paste was used for patch testing. The patch test dilution was high; no serial dilutions were undertaken. No control tests are reported. Purity of the test substance is not disclosed. The information does not confirm that Pigment Red 57 itself was the cause of the reaction.

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Pigment Red 57

Not applicable

Due to the limited quality of the submitted toxicological data, a NOAEL/LOAEL cannot be derived with sufficient confidence from these studies, and therefore a MOS cannot be calculated for Pigment Red 57.
However, the systemic exposure dose (SED) has been calculated to be 0.003 mg/kg bw/d for a typical non-oxidative hair dye formulation, which is considered very low by the SCCS and is more than 30.000 fold lower than the identified effect level of 100 mg/kg bw/day in female rats.

Therefore, the SCCS considers that it is unlikely that there would be a safety concern for consumers from the use of Pigment Red 57 when used as a direct dye in non-oxidative hair dye formulations at a maximum on-head concentration of 0.4%.

3.3.14. Discussion

Physico-chemical properties
Pigment Red 57 is used as a "semi-permanent" hair dye at a maximum concentration of 0.4% in the finished cosmetic formulation.
Pigment Red 57 was described to be stable in a cosmetic formulation for 7 months (recovery 95%). However, no supporting data was provided.

Irritation, sensitisation
A 10% preparation of Pigment Red 57 caused mild irritation to the skin and eyes of rabbits. The test item was a slight irritant at the test concentration of 1% in water in an HET-CAM study. From the results of the local lymph node assay it is concluded that Pigment Red 57 is not a skin-sensitiser. A single case report in man does not confirm that Pigment Red 57 itself was the cause of an allergic reaction.

Dermal absorption
Only 4 chambers were considered available (3 donors) for calculating absorption. However, Pigment Red 57 was below the LOD in all samples. Therefore, based on the LOD a maximum absorption of 0.029 $\mu g/cm^2$ of Pigment Red 57 from a direct hair dye containing 0.4% of Pigment Red 57 was determined (receptor fluid + lower skin + upper skin). Despite the limitations of this study, which would ordinarily have excluded the results from further consideration, use of the limits of detection of Pigment Red 57 provides an acceptable estimation of potential absorption.

General toxicity
Pigment Red 57 (Lithol Rubine B and BK) is of low acute toxicity in rats and dogs. Repeated and chronic oral administration to rodents showed the kidney as a primary target organ in the high doses. However, the SCCS considers that the toxicological data are for most of them not complying with current standards and not fully reported. Most of the studies provided in the dossier are indeed summaries from the BIBRA's report from 1997. These studies have also been evaluated recently by the EFSA panel on food additives and nutrient sources added to food. No NOAEL can be derived from the submitted studies. However the LOAEL is probably around 100 mg/kg bw/d.

Mutagenicity
Overall, the genotoxicity of Pigment Red 57 is sufficiently investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Pigment Red 57 did not induce gene mutations in bacteria either without metabolic activation or with Aroclor induced rat liver S9-mix or uninduced hamster liver S9-mix. Pigment Red 57 did not induce gene mutations or chromosomal aberrations (structural or numerical) in mammalian cells. There was no induction of micronuclei in an *in vivo* micronucleus test in mice. Consequently, Pigment Red 57 can be considered to have no genotoxic potential and additional tests are unnecessary.

Carcinogenicity
Pigment Red 57 has been studied in long term carcinogenicity experiments after oral administration of mice and rats and skin application of mice. Due to the inadequacy of the studies, no conclusions concerning potential carcinogenic effects of Pigment Red 57 can be drawn from the experiments.

Systemic exposure

The systemic exposure dose calculated following percutaneous absorption of 0.4% Pigment red 57 in a typical direct hair dye formulation is 0.003 mg/kg bw/d which is considered very low by the SCCS.

4. CONCLUSION

Despite some shortcomings in the toxicological dataset, the SCCS considers that the available data does not indicate toxic effects of Pigment Red 57 at the low systemic exposure level foreseen after its use as a hair dye due to low absorption. Therefore, the SCCS concludes that Pigment Red 57 does not pose a risk to the health of the consumer, when used as a direct dye in non-oxidative hair dye formulations, at a maximum on-head concentration of 0.4%.

However, Pigment Red 57 is also used in other cosmetic products as a colorant leading to additional exposure of the consumers. The SCCS recommends that the safety of Pigment Red 57 for this use should be assessed.

5. MINORITY OPINION

Not applicable

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