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

CONCERNING

PIGMENT RED 57

COLIPA n° : /

Adopted by the SCCNFP during the 28th plenary meeting
of 25 May 2004

1. Terms of Reference

1.1 Context of the question

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

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is Pigment Red 57 safe for use as a hair dye ingredient?

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

1.3 Statement on the toxicological evaluation

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

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

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

2. Toxicological Evaluation and Characterisation

2.1. General

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.

2.1.1. Primary name

Pigment Red 57 (INCI name)

2.1.2. 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

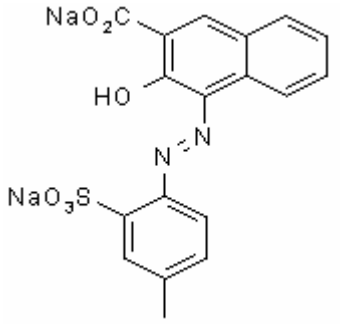
2.1.3. Trade names and abbreviations

Trade name	:	Rouge Covanor W 3604 (LCW)
Other names	:	Lithol Rubin B; Lithol Rubin BK (calcium salt); Japan Red 201; D&C Red Nº 6
COLIPA n°	:	/

2.1.4. CAS n° / EINECS n° / Colour Index n°

CAS n°	:	5858-81-1
EINECS n°	:	227-497-9
Colour Index	:	CI 15850

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula	:	$C_{18}H_{14}N_2O_6S \cdot 2Na$
Mol weight	:	430.35 g/mol

2.1.7. Purity, composition and substance codes

All analytical data are related to batches B2095 and Lot 7 CO2

Purity

Titre as determined by HPLC	:	98 % (qualitative) (254 nm)
Titre as determined by NMR	:	96.2 % quantitative)
Water content	:	0.56 % (w/w)
Ash content (sulphated)	:	34.6 %
Heavy metals	:	< 114 ppm

Potential impurities

2-Amino-5-methylbenzene sulfonic acid, sodium salt	:	< 0.2 %
3-Hydroxy-4-(4-methylphenylazo) -2-naphthalene carboxylic acid	:	< 0.5 %
3- Hydroxy-2-naphthalenecarboxylic acid, sodium salt	:	< 0.4 %
p- Toluidine	:	< 15 ppm

2.1.8. Physical properties

Appearance	:	Orange-red powder
Melting point	:	348.9 ºC (calculated)*
Boiling point	:	791 ºC (calculated)*
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	4.25 E- 21 (calculated)*
Log P_{ow}	:	3.587 ± 0.796 (calculated)*

* See General Comments below

2.1.9. Solubility

1.0% soluble in water (pH 8.1), 0.38% soluble in acetone/water (1:1), 0.5% soluble in DMSO, < 0.3% soluble in ethanol/water (4:6).

2.1.10. Stability

Stable in water (0.5%) at room temperature during 7 days.
Stable in a cosmetic formulation for 7 months at 25ºC (recovery: 95%).

General comments on analytical and physico-chemical characterisation

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

* Since log P_{ow} is known to strongly depend on the pH, the reported value 3.6 ± 0.8 is not helpful if the pH conditions used in the calculation are unknown or not related to physiological conditions and to the pH conditions of the percutaneous absorption studies.

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

2.2. Function and uses

Pigment Red is used as a “semi-permanent” hair dye at a maximum concentration of 0.4% in the finished cosmetic formulation. It is intended for weekly use with typical applications of 35 ml.

TOXICOLOGICAL CHARACTERISATION

The mono-azo-dye (mainly as calcium-salt) is approved as a food colorant and used already for a long time. Thus numerous toxicological studies are available in reviews mostly originated in the late 1980s and early 1990s; these studies include also long-term toxicity and are presented for either the sodium or the calcium-salt.

Overviews on the toxicological profile for both salts are given in the BIBRA Toxicity Profiles for Lithol Rubine B (Na-salt, = Pigment Red 57) and Lithol Rubine BK (Ca-salt), as well as in “Kosmetische Färbemittel”, DFG 1991 (References 2, 3, 4).

The dye is approved as Ca-salt (E 180) for food use (treatment of cheese rind) in the EU and as sodium and calcium salt for general use in cosmetics. In the US both salts are approved for usage in drugs and cosmetics (with the exception of eye area use). Similar, in Japan both salts are approved for OTC-drugs and cosmetics for external application. According to FDA a maximum daily dose of 5 mg is considered to be safe and a group ADI of 0.15 mg/kg bw/day was established (FDA 1982). In the EU an ADI of 0-1.5 mg/kg was deduced by the SCF in 1983 (EG Doc III/9280/90). (Ref. 1, 2, 3, 4)

Moreover the applicant of the new dossier carried out a literature search using the ChemI Plus-system, a reputable search system including most of the acknowledged data bases as MEDLINE, TOXNET NLM Gateway etc. A statement is given that all hits of relevance for risk assessment of Pigment Red 57 were included in the present safety evaluation. Thus, the description of the results obtained are sufficient for an evaluation of the general toxicology of Pigment Red 57 as for the intended use and applied concentrations.

2.3. Toxicity

2.3.1. Acute oral toxicity

Rat
LD_{50} > 10,800 mg/kg bw (Na-salt)
LD_{50} > 9,800 mg/kg bw (Ca-salt)
LD_{50} > 5,000 mg/kg bw (Ca-salt)

Dog
LD_{50} > 9,800 mg/kg bw (Ca-salt)

Based on the observed high LD_{50} values of ≥ 5000 mg/kg bw, both salts and consequently *Pigment Red 57* are evaluated to be of low acute oral toxicity.

Ref.: 2, 3

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

No data

2.3.5 Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Subchronic oral toxicity

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.: 3

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: 3

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 %).

2.3.8. Sub-chronic dermal toxicity

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.

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

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.: 3

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.: 2

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.: 2

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Guidelines	:	/
Species/strain	:	New Zealand albino rabbit
Group size	:	3 animals (sex not indicated)
Test item	:	Pigment Red 57, 10% dilution in propylene glycol
Batch no.	:	/
Dosages	:	1 ml
GLP	:	/

Pigment Red 57 was investigated as a 10% dilution in propylene glycol for its comedogenic and 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 follicular keratosis and irritation.

Results

No indication was found that Pigment Red 57 might cause skin irritation under the described test conditions. The noted grade of irritation after the repeated open application was 0. In addition, no significant increase in follicular keratosis was noted (Grade 1). Thus Pigment Red 57 is not expected to be comedogenic.

Ref.: 5

2.4.2. Irritation (mucous membranes)

Guidelines	:	/
Species/strain	:	Albino rabbit, strain no given
Group size	:	6 or more animals (sex not indicated)
Test substance	:	Pigment Red 57 (lake)
Batch no.	:	/
Dosages	:	20 mg (equal to 0.2 ml of a 10% aqueous solution, twice daily)
GLP	:	/

Pigment Red 57 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 Pigment Red 57 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 almost no irritative effects even immediately after application. 24 hours after all applications of the 10% solution, no indications for eye irritation were noted at any scoring throughout the entire study period.

Ref.: 6

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 No	:	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.

2.5. Sensitisation

Local Lymph Node Assay (LLNA)

Guideline	:	OECD 429 (2000)
Species/strain	:	Mice CBA/J
Group size	:	5 females
Test substance	:	Pigment Red 57
Batch No	:	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

The skin sensitising potential of Pigment Red 57 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 Pigment Red 57 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.

Animals were checked twice daily for morbidity/mortality. Observation for clinical signs was done daily before and at least once after dosing. Bodyweight was determined at day – 1 and day 5.

At day 5 the mice received an intravenous injection of 250 µl phosphate buffered saline containing 21.4 µCi of [H^3] methyl thymidine. About five hours later the mice were sacrificed by CO_2-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 [H^3] 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
Neither clinical signs nor mortalities were observed throughout the study period. The body weight development was not affected by the treatment.

The weight of lymph nodes did not increase compared to the vehicle controls due the treatment with Pigment Red 57. In addition, the mean stimulation indices were also 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 (all figures were well below the trigger value of three), no indication was found that Pigment Red 57 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, Pigment Red 57 did not reveal any potential to be a skin sensitiser if tested with two different vehicles up to 4 % (exceeding the maximum solubility). The concurrent positive control demonstrated the validity and sensitivity of the assay. Based on these findings Pigment Red 57 is evaluated not to be a skin-sensitiser.

Ref.: 8

2.6. Reproductive Toxicity / Teratogenicity

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.: 2

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.: 2

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.: 3

Furthermore in reference 3 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.

2.7. Toxicokinetics (incl. Percutaneous Absorption)

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 No	:	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.

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.

Ref.: 9

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Assay

Guideline	:	OECD 471 (July 1997)
Species/strain	:	*S. typhimurium* TA 98; TA 100; TA 102; TA1535; TA 1537
Test substance	:	D&C RED NO.6 W 003
Batch number	:	B2095 (LCW)
Lot number	:	AK8999
Purity	:	HPLC value
Concentrations	:	1-5000 µg/plate (5 doses); 30-3000 µg/plate (5doses); in the 2nd experiment with TA 102 different doses were employed.
Replicate	:	2 experiments; 3 plates/dose
Positive controls	:	according to the guidelines
Metabolic act.	:	Aroclor 1254 induced rat liver homogenate (purchased; not checked)
GLP	:	in compliance

Results
Toxicity: no information

Mutagenicity: no increase in the number of revertants was observed in 4 strains; on TA 102 a small increase in the number of revertants was observed, but it was without a biological significance.

The test item is not mutagenic on *Salmonella typhimurium* reverse mutation assay.

Conclusion
Pigment Red 57 is not mutagenic on bacterial cells.

Ref.: 10

In a published paper the test item is not mutagenic in strains TA 1535, 1537, 1538, 98, 100.

Ref.: 11

In Vitro Mammalian Cell Gene Mutation test

Guideline	:	OECD 476 (July 1997)
Species/strain	:	Mouse Lymphoma L5178Y (Thymidine kinase locus)
Test substance	:	RED 201 WR 21176
Batch number	:	/
Lot number	:	7 CO2 (Fa. Toshiki Japan)
Purity	:	95.9 weight % (NMR spectrum)
Concentrations	:	10-320 µg/ml with and without S9 Treatment 4 hours: 1st experiment Treatment 24 hours: 2nd experiment (only without S9)
Replicate	:	2 cultures/treatment
Metabolic act.	:	Non induced rat liver homogenate
Positive controls	:	MMS: without S9; DMNA: with S9
GLP	:	in compliance

Results
Toxicity: doses from 39.1 to 5000 µg/ml (-S9) and from 39.1 to 4750 µg/ml (+S9) were evaluated for toxicity. After 4 hours of treatment a strong toxic effect was noted at 312.5 µg/ml in the absence of S9: this dose was used as the maximum dose. No osmolality was detected at this dose.

Mutagenicity: The two positive controls, MMS and DMNA, induced small colony mutants (clastogenic effect), DMNA induced large colony mutants (gene mutation effect) only in one culture. In the 24 hours of treatment (-S9) MMS induced both small and large colony mutants. There is some inadequacy in the performance of the experiment, due to the use of these specific positive controls (DMNA is not suggested by the OECD guideline).
The historical data indicate only the frequency of the mutant colonies for both the positive controls. There is no indication of a statistically increase of the mutant colonies of different sizes induced by the test item.

Conclusion
Pigment Red 57 is not active for the induction of gene mutations in this particular assay.

Ref.: 12

2.8.2. Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus test

Guideline	:	OECD 474 (July 1997)
Species/strain	:	NMRI mice

Test substance	:	RED 201 WR 21176
Batch number	:	/
Lot number	:	lot 7 CO2 (Fa.Toshiki Japan)
Purity	:	95.9 weight% (NMR spectrum)
Dose levels	:	500, 1000, 2000 mg/kg for 24 hours; 2000 mg/kg for 48 hours 6 M + 6 F per group
Treatment	:	oral
Positive control	:	CPA, 40 mg/kg, oral, 24 hours
GLP	:	in compliance

Results
Toxicity: a dose of 2000 mg/kg was administered to 4 animals (2 males and 2 females) and observed for 48 hours. There was no sign of toxicity.

Mutagenicity: CPA induced 32 and 23.8 MN per 2000 PCE respectively in males and in females; the vehicle treated animals (CMC 0.5 %) showed 1.0 and 1.6 MN per 2000 PCE respectively in males and females.
The respective data for the test item treated animals were:500 mg/kg = 0.0 and 0.6; 1000 mg/kg = 1.2 and 2.2; 2000 mg/kg = 0.8 and 0.4 (24 hours); 2000 mg/kg = 0.8 and 0.6 (48 hours).
The PCE per 2000 cells was not reduced in the bone marrow of the treated animals, compared with the vehicle treated animals, thus not indicating that the substance has reached the target cells.
Therefore the study is not adequate and unsuitable for the evaluation.

Conclusion
The test item has been inadequately tested in the *in vivo* test for the induction of Micronuclei.

Ref.: 13

2.9. Carcinogenicity

Oral studies (sodium salt)

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. Gross examination of all of the mice and complete microscopic examination of (unspecified) tissues in the high-dose group revealed an increased incidence of alveolar adenomas in the high dose males.

Ref.: 2

Rats
Group of 70 male and 70 female rats (derived from parents similarly exposed for 60 days before mating and throughout pregnancy and lactation) were fed 0.05, 0.3 or 2 % [roughly 25, 150 or 1000 mg/kg bw/day] of *Pigment Red 57* in the diet for up to 2 years. Microscopic examination was carried out of all major organ tissues in the high-dose groups. There was a slight increase in the incidence of Leydig cell adenomas in the high dose males (6% as opposed to 2% in the controls).

Ref.: 2

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.: 3

2.10. Special investigations

No data

2.11. Safety evaluation

Not applicable

2.12. Conclusions

Pigment Red 57 (Lithol Rubine B and BK) is of low acute toxicity in rats and dogs. Repeated and chronic oral administration to rodents up to 2 years, showed the kidney as a primary target organ in the high doses. The lowest NOAEL (150 mg/kg/bw/day) was obtained in the 2 years toxicity study in rats performed with the F1-generation of parent-animals pretreated at the same dose levels. The SCF also used this study to derive an ADI for this compound.

There is no indication for corrosivity or comedogenic effects on the skin. Also no irritation on mucous membranes was noted. From the results of the local lymph node assay it is concluded that Pigment Red 57 is not a skin-sensitiser.

There was no convincing evidence of reproductive effects in rats and rabbits treated orally. Pigment Red 57 (Lithol Rubine B) did not cause skin irritation in rabbits but induced skin sensitisation in man. Diluted Lithol Rubine BK produced little irritation in man and rabbits and failed to induce sensitisation in man.

Pigment Red 57 has been tested for the induction of gene mutations in bacterial cells and in the mammalian cells and found negative. The *in vivo* study for the induction of the micronuclei is inadequate for the evaluation.

None of the available carcinogenicity studies are reported in sufficient details to allow an evaluation of the potential carcinogenicity of Pigment Red 57.

2.13. References

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3. Opinion of the SCCNFP

The SCCNFP is of the opinion 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.

4. Other Considerations

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5. Minority opinions

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