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

CONCERNING

ACID RED 92

COLIPA n° C53

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

1. Terms of Reference

1.1 Context of the question

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

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is Acid Red 92 safe for use in cosmetic products as a hair dye ingredient?

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

1.3 Statement on the toxicological evaluation

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

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

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

2. Toxicological Evaluation and Characterisation

2.1. General

Acid Red 92 is listed as CI 45410 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. Other limitations and requirements : not more than 1 % 2-(6-hydroxy-3-oxo-3*H* xanthen-9-yl) benzoic acid and 2 % 2-(bromo-6-hydroxy-3-oxo-3H-xanthen-9-yl) benzoic acid.

2.1.1. Primary name

Acid Red 92 (INCI)

2.1.2. Chemical names

- 3,4,5,6-Tetrachloro-2-(1,4,5,8-tetrabromo-6-hydroxy-3-oxoxanthen-9-yl)benzoic acid, disodium salt
- 2',4',5',7'-Tetrabromo-4,5,6,7-tetrachloro-fluorescein,disodium salt
- Fluorescein, 2',4',5',7'-tetrabromo-4,5,6,7-tetrachloro-, disodium salt (CA index name 8CI)
- Spiro[isobenzofuran-1(3H), 9'-[9H]xanthen]-3-one", 2'4',5',7'-tetrabromo-4,5,6,7-tetrachloro-3',6'-dihydroxy, disodium salt (CA index name 9CI)

Comment
The Chemical/IUPAC name of Acid Red 92 in the EU inventory refers to the free acid (not to its disodium salt).

2.1.3. Trade names and abbreviations

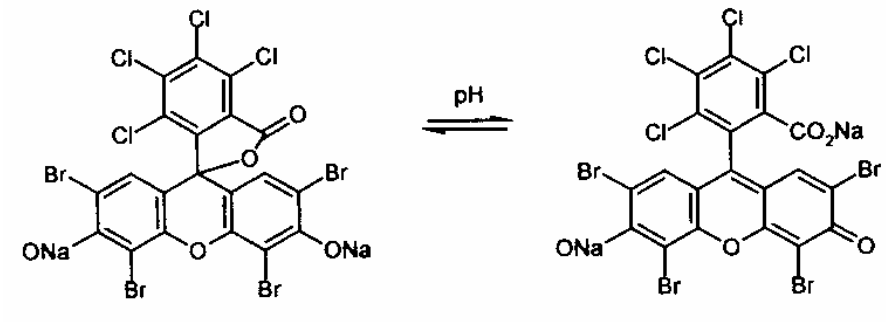
COLIPA n^{o}	:	C 53
Trade name	:	Bromo De Phloxine 27 (LCW); Gardex Fuchsia Red (Wella), WR802177
Other names	:	Phloxine B D&C Red No. 28 Eosin Blue Cyanosine Japan Red 104

2.1.4. CAS / EINECS / COLOUR INDEX number

CAS	:	18472-87-2
EINECS	:	242-355-6
Colour Index	:	CI 45410

The dye contains 3 additional isomers with CAS numbers: 13473-26-2, 84473-86-2 and 94021-88-2.

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{20}H_2Br_4Cl_4O_5Na_2$
Mol weight : 829.66 g/mol

2.1.7. Purity, composition and substance codes

Substance code : A 005939 (Gardex Fuchsia Red)
Batches used : AR92-020420 (Gardex Fuchsia Red); ref. standard R0071

The information provided by the submission report (ref. 1a), a GLP study (ref. 1b) and by FDA and Japan specifications is summarized in the following table.

	Ref. 1a	*Ref. 1b*	*FDA and Japan*
Purity by NMR (quantitative) by potentiometric titration by quantitative HPLC (external standard)	> 83% – – –	 84.8 % 86.5 % 85.6 %	> 85% – – –
Relative chromatographic purity (HPLC - UV/VIS peak area method)	>94% at 254 nm	94.6% at 210 nm 95.2% at 254 nm 99.4% at 548 nm	
Loss on drying (vacuum dessication)	3 - 8 %	6.1 %	<10% (at 135°C)
Sum of volatile matter and chlorides and sulfates (calc. as sodium salts)	–	–	< 15%
Water content (Karl-Fischer)	7-12 %	9.6 %	
Water insoluble matter (alkaline solution)	–	–	< 0.5%
Sulfated Ash content	13 – 18 %	16.9 %	
Bromide	< 1 %		
Iodide	< 150 ppm		
Lead	< 20 ppm		< 20 ppm
Mercury	< 1 ppm		< 1 ppm
Arsenic	< 3 ppm		< 3 ppm
Iron	< 100 ppm		
A tribromo-homologue of Acid Red 92	Not possible to be quantitated	4.7% at 210 nm 4.3% at 254 nm 0.3% at 548 nm	–
Tetrachlorophthalic acid	–	–	< 1.2%
Brominated resorcinol	–	–	< 0.4%
2,3,4,5-Tetrachloro-6-(3,5-dibromo-2,4-	–	–	

diyhdroxybenzoyl)benzoic acid			< 0.7%
2',4',5',7'-Tetrabromo-4,5,6,7-tetrachlorofluorescein, ethyl ester	–	–	< 2%

Solvent Residues
Solvents such as methanol, isopropanol, n-propanol, acetone, ethyl acetate, cyclohexane, methyl ethyl ketone and monochlorobenzene were not detected.

2.1.8. Physical properties

Appearance : Red powder
Melting point : > 300 °C *
Boiling point : 850 °C (calculated by QSAR) *
Density : /
Rel. vap. dens. : /
Vapour Press. : /
Log P_{ow} : 9.99 (acid, calculated ACD) *
pKa : 4.29 (acid, most acidic, calculated ACD) *

* See General Comments below

2.1.9. Solubility

Soluble in water : > 10 w% (pH 8.5)
acetone : 9.9 w% (pH 8.9)
DMSO : > 10 w%

2.1.10 Stability

Stability in water (5% w/v; pH 7.3) very good during 7 days at room temperature
in water/acetone (1:1, 5% w/v) very good during 7 days at room temperature
in DMSO (7% w/v) very good during 7 days at room temperature

Stability data on a common market formulation are not acceptable.

General comments on analytical and physico-chemical characterisation

* The information provided on the compound is largely incomplete, confusing and controversial, not conforming with SCCNFP Notes of Guidance. Different values are reported by different sources for the same batch (see table in section 2.1.7), and as a result, the purity of the test substance in most toxicological studies is reported as 94.0 - 99.5% or 99.5 area % (HPLC) instead of the true value of 84.8 % (by NMR).

* Tetrachlorophthalic acid, Brominated resorcinol, 2,3,4,5-Tetrachloro-6-(3,5-dibromo-2,4-diyhdroxybenzoyl)benzoic acid, and 2',4',5',7'-Tetrabromo-4,5,6,7-tetrachlorofluorescein, ethyl ester were identified and quantitated by FDA only, while in the submission's reports (ref.1a-b) only a tribromo-homologue of Acid Red 92 of unknown content is reported and another 9 impurities, of unknown nature, are detectable by HPLC-UV/VIS .

* The physical properties has been calculated without indicating the method used. Furthermore, calculated values can not be accepted as estimates of the true physical constants without justification, indicating that the reported values are realistic (possible decomposition of the test substance at elevated temperatures).
* Although the molecule contains two ionisable groups, only one pKa is reported, followed by the ambiguous specification "acid, most acidic, calculated ACD".
* Since log P_{ow} is known to strongly depend from the pH, the reported value 9.99, followed by the ambiguous specification "acid, calculated ACD", seems to correspond to an acidic pH, well below pKa= 4.29, i.e. not related to physiological conditions and to the pH conditions of the percutaneous absorption studies.
* The reported stability data on a common market formulation are based on a single determination and comparison of the result with a "theoretical" content" (whatever this means); this is not an acceptable stability test.
* No information is provided on the content of 2-(6-hydroxy-3-oxo-3H xanthen-9-yl) benzoic acid and 2 % 2-(bromo-6-hydroxy-3-oxo-3H-xanthen-9-yl) benzoic acid, which are restricted in the dye according to Cosmetic Directive.

2.2. Function and uses

Acid Red 92 is intended for use in semi-permanent hair dye formulations as a direct dye at a maximum concentration of 0.4% and in oxidative hair dyes at a maximum final concentration of 2.0 % after mixing with 1.5 volume parts of a developer-mix (5 % in the formulation).

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

No data

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

Guideline	:	/
Purpose	:	to select dose levels for a subsequent 13-wk study
Species/strain	:	Rat, HanBrl: WIST(SPF)
Group size	:	5 males + 5 females
Batch no	:	AR92-020420
Purity	:	94-99.5 area% (HPLC)

Dose	:	0, 10, 50 and 250 mg/kg bw/day
Vehicle	:	Water (10 ml/kg bw/day)
Exposure period	:	4 weeks (7 days per week)
GLP	:	/

Results

Mortalities	:	none
Clin. signs	:	reddish faeces in all test groups (dose related severity)
Body weight	:	no treatment-related changes
Food intake	:	no treatment-related changes
Haematology	:	percentage of basophils decreased in high-dose females platelet count increased in high-dose females
Organ weights	:	no treatment-related changes
Pathology	:	stomach irritation in both sexes at 50 and 250 mg/kg bw/day (focal spongiosis of the limiting ridge in high-dose group both sexes and dyskeratosis in mid- and high dose females).

Conclusion
10, 50 and 250 mg/kg bw/day were selected as dose levels for a subsequent 13-wk study.

Ref.: 2

2.3.5 Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Subchronic oral toxicity

Guideline	:	OECD 408 (1998)
Species/strain	:	Rat, HanBrl: WIST(SPF)
Group size	:	10 males + 10 females
Batch no	:	AR92-020420
Purity	:	94.6 -99.4 area% (HPLC)
Dose	:	0, 10, 50 and 250 mg/kg bw/day
Vehicle	:	Water (10 ml/kg bw/day)
Exposure period	:	13 weeks (7 days per week)
GLP	:	In compliance

Results

Mortalities	:	one male and one female of the mid-dose group died, probably as a result of dosing errors.
Clin. signs	:	reddish faeces in all test groups
Detail. obs/FOB	:	myosis in high-dose males and females decreased locomotor activity in high-dose males
Ophthalmoscopy	:	no treatment-related changes
Body weight	:	no treatment-related changes
Food intake	:	no treatment-related changes

Haematology	:	increased absolute eosinophils count in high-dose females
Clin. chemistry	:	decreased triglycerides, protein levels and ALAT activity in high-dose males
Urinalysis	:	impaired concentrating ability (increased volume and decreased density) in high-dose males and females. Increased urinary pH in high-dose males. Reddish urine in mid- and high-dose groups.
Organ weights	:	no treatment-related changes
Macroscopy	:	passive discolouration of various segments of the digestive tract in all groups
Pathology	:	stomach irritation in both sexes at 250 mg/kg bw/day (vacuolation limiting ridge epithelium, hyaline inclusions in glandular mucosa and squamous hyperplasia in most males and females, and submucosal cell infiltrate in all females). In males of the 50 mg/kg bw/day group slight stomach irritation was observed (squamous hyperplasia) in a number of males.

Conclusion
10 mg/kg bw/day was established by the authors as the NOAEL.

Remark
Assuming that in the mid-dose group the signs of stomach irritation represent a local effect due to irritating potential of the test substance, and assuming that the mortalities were due to dosing errors, the NOAEL for systemic toxicity could be placed at 50 mg/kg bw/day.

Ref.: 3

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No data

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Guideline	:	OECD 404 (1992)
Species/strain	:	New Zealand albino white rabbit
Group size	:	3 animals (1 male, 2 females)
Observ. Period	:	14 days
Test substance	:	Acid Red 92, moistened with 0.1ml of water
Purity	:	94.0 - 99.5%
Batch no	:	AR92-020420
Dose level	:	0.5 g (4h contact under semi-occlusion)

GLP : in compliance

The primary skin irritation potential of Acid Red 92 was investigated by topical semi-occlusive application of 0.5 g to the intact left flank of each of three young adult New Zealand White rabbits. The duration of the treatment was four hours. The scoring of skin reactions was performed 1, 24, 48 and 72 hours, as well as 7, 10 and 14 days after removal of the dressing.

Results
The test item did not elicit any skin reactions at the application site of any animal (all scores = 0). The individual mean score for erythema/eschar and oedema for each of the three animals was 0.
A light to marked red staining was apparent in all animals for the majority of the observation period and was still present in one animal 14 days after removal of the dressing, the end of the observation period for all animals. No corrosive effects were noted on the treated skin of any animal at any of the measuring intervals.

Conclusion
Based upon the referred classification criteria (Commission Directive 2001/59/EC), Acid Red 92 is considered to be not irritating to skin.

Ref.: 4

2.4.2. Irritation (mucous membranes)

Guideline	:	OECD 405 (1987)
Species/strain	:	New Zealand albino white rabbit
Group size	:	3 animals (1 male, 2 females)
Observ. Period	:	17 days
Test substance	:	Acid Red 92, moistened with 0.1ml of water
Purity	:	94.0 - 99.5%
Batch no	:	AR92-020420
Dose level	:	100 mg
GLP	:	in compliance

The primary eye irritation potential of Acid Red 92 was investigated by instillation of 0.1 g into one eye of three young adult New Zealand White rabbits. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours, as well as 7 and 10, 14 and 17 days after test item application.

Results
The mean score was calculated across 3 scoring times (24, 48 and 72 hours after instillation) for each animal for corneal opacity, iris, redness and chemosis of the conjunctivae, separately.

The individual mean scores were as follows:

$<S_{\text{cornea opacity}}>$	=	0.0 - 0.0 - 1.0
$<S_{\text{iris}}>$	=	0.0 - 0.0 - 0.0
$<S_{\text{conjunctiva redness}}>$	=	2.0 - 2.0 - 2.0
$<S_{\text{conjunctiva chemosis}}>$	=	1.3 - 1.3 - 1.3

The instillation of the test item into the eye resulted in mild to moderate, early-onset and transient ocular changes, such as reddening of the conjunctivae and sclerae, discharge and chemosis. A slight corneal opacity, affecting up to the whole area of the cornea, was also apparent in one animal from 24 hours to 10 days after treatment, A light to marked red staining was also observed in the treated eye of all animals during the first 7 days after treatment. All ocular effects were reversible and were no longer evident 17 days after treatment. No abnormal findings were observed in the iris of any animal at any reading. No corrosion was observed at any of the measuring intervals.

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

Ref.: 5

2.5. Sensitisation

Local Lymph Node Assay

Guideline	:	OECD 429 (2000)
Species/strain	:	CBA/J mouse
Group size	:	4 females / dose group
Observ. Period	:	6 days
Test substance	:	Acid Red 92
Purity	:	94.0 - 99.5%
Batch no	:	AR92-020420
Dose levels	:	control, 0.5%, 1.5%, 3.0% and 7.0% (w/v) in DMSO; 7% reaching the solubility limit in the vehicle; reliability check with o-phenylenediamine described
GLP	:	/

Acid Red 92 was tested in different concentrations (0.5, 1.5, 3.0, 7.0 % (w/v)) in DMSO (vehicle). On days 0, 1 and 2 the animal received 25 µl of the test item formulation, positive control or vehicle on the dorsal surface of each pinnae. Each dose was tested on one animal group, which consisted of 5 animals.
Morbidity/mortality checks were performed twice daily. Clinical examinations were performed daily. Individual body weights were recorded on days - 1 and 5. All animals were sacrificed on day 5 for assessment of cell proliferation.

Results
No mortality was observed during the study. There were no treatment-related clinical signs. There were no treatment-related effects on body weight or body weight gains.
The positive control (p-phenylenediamine) induced a positive response, as it elicited at least a 3-fold increase in isotope incorporation relative to the vehicle. The mean stimulation index was 3.9 at the concentration of 1%.
The test substance induced a negative response, as it did not elicit at least a 3-fold increase in isotope incorporation relative to the vehicle. The mean stimulation indices were 0.9, 1.3, 1.5 and 1.9 at the concentrations of 0.5 %, 1.5%, 3.0% and 7.0%, respectively.

Conclusion
Based on these results, the test substance is not a skin sensitizer under the defined experimental conditions. The experimental conditions used in this study have been stricter than use conditions. It is therefore concluded that Acid Red 92 does not pose a sensitizing risk to consumers when used as intended.

Ref.: 6

2.6. Teratogenicity

Prenatal developmental toxicity study, range-finding study

Purpose	:	to select dose levels for a subsequent prenatal developmental toxicity study
Species/strain	:	Rat, HanBrl: WIST(SPF)
Group size	:	5 females (mated)
Batch no	:	AR92-020420
Purity	:	94.6-99.4 area% (HPLC)
Dose levels	:	0, 10, 50 and 250 mg/kg bw/day
Vehicle	:	water (10 ml/kg bw/day)
Treatment period	:	days 6-20 of gestation

Results

Clinical signs	:	reddish faeces in all test groups
Body weight gain	:	slightly reduced at 250 mg/kg bw/day
Food intake	:	slightly reduced at 250 mg/kg bw/day from days 6-15 p.c.
Reproductive data	:	no treatment related effects
General foetal data	:	no treatment related effects

Conclusion
10, 50 and 250 mg/kg bw/day were selected as dose levels for a subsequent prenatal developmental toxicity study

Ref.: 7

Prenatal developmental toxicity study, main study

Guideline	:	OECD 414
Species/strain	:	rat, HanBrl: WIST(SPF)
Group size	:	24 females (mated)
Batch no	:	AR92-020420
Purity	:	94.6-99.4 area% (HPLC)
Dose levels	:	0, 10, 50 and 250 mg/kg bw/day
Vehicle	:	water (10 ml/kg bw/day)
Treatment period	:	days 6-20 of gestation
GLP	:	in compliance

Results

Mortality	:	no test-substance related effects. 1 low- and 1 mid-dose female died due to dosing error
Clinical signs	:	reddish faeces in all test groups

Body weight gain	:	transient slight reductions at 250 mg/kg bw/day
Food intake	:	transient slight reductions at 250 mg/kg bw/day
Reproductive data	:	no treatment related effects
Necropsy F0	:	reddish stomach/ intestinal content in mid- and high-dose group
General foetal data	:	no treatment related effects
Foetal visceral exam.	:	no treatment related effects
Foetal skeletal exam.	:	no treatment related effects

Conclusion
The test substance elicited slight maternal toxicity at 250 mg/kg bw/day but was not embryo-toxic or teratogenic at the doses tested. The NOAEL for maternal toxicity was considered to be 50 mg/kg bw/day.

Ref.: 8

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Percutaneous absorption, study 1

Guideline	:	/
Test system	:	Split thickness pig skin (1000 µm), 5 samples. No justification for the use of 1000 µm (= full thickness) skin is given
Contact time	:	30 minutes
Test substance	:	Acid Red 92 (WR802177), used at 1.67% (final) in a an oxidative formulation (code 8190568C, composition not stated)
Control	:	composition not stated
Purity	:	94.0 - 99.5%
Batch no	:	/
Application	:	100 mg/cm²
Receptor fluid	:	0.14M NaCl, 2mM K_2HPO_4, 0.4mM KH_2PO_4, 100 IU penicillin/ml, 97µg streptomycin/ml, 3% ethanol (pH not stated)
GLP	:	in compliance

The cutaneous absorption of Acid Red 92 was determined in a representative hair dye formulation containing 1.67% of the test substance using pig skins *in vitro*. A dose of 400 mg formulation was applied on skin samples (1670 µg Acid Red 92 /cm^2 pig skin) for 30 minutes and subsequently rinsed off with water and shampoo. After 72 hours, the amount of the test substance was determined in the receptor fluid, in the skin extracts (epidermis and upper dermis separated) and in the rinsing solution using HPLC analysis.

Results
The content of Acid Red 92 in all fractions in the receptor fluid was below 119 ng/cm^2 adding up all 6 fractions. The content of all fractions was below the limit of quantification of 20 ng/cm^2 for each single fraction. Considering the limit of quantification as upper limit, the amount of Acid Red 92 in the receptor fluid was < 0.119 µg/cm^2 (or < 0.007% of the applied dose). Correspondingly, the amount of < 0.119 µg/cm^2 was regarded as to have passed the skin barrier during the experimental period of 72 hours.
The concentrations of Acid Red 92 detected in the separated skin layers were 2.607 ± 1.304 µg/cm^2 (or 0.156 ± 0.078%) in the epidermis, and < 0,046 µg/cm^2 (or < 0.003%) in the upper dermis. A total recovery of 98.6 % was calculated, including the amount of test substance in the rinsing solution (1640 µg/cm^2 or 98.4 %).

Conclusion
The laboratory concludes that, under the described test conditions, that correspond to realistic in use conditions, a dermal penetration rate of < 0.119 µg/cm^2/72h was obtained.
However, adding the receptor fluid, epidermis and dermis values generates a percutaneous absorption value for Acid Red 92 of 2.772 µg/cm².

Ref.: 9

Percutaneous absorption, study 2

Guideline	:	/
Test system	:	Full thickness pig skin (1000 µm), 5 samples. No justification for the use of full thickness skin is given
Contact time	:	30 minutes
Test substance	:	Acid Red 92 (WR802177, red powder), used at 1.67% in DMSO
Control	:	DMSO
Purity	:	94.0 - 99.5%
Batch no	:	AR92-020420
Application	:	100 µl/cm² (≈ 100 mg/cm²)
Receptor fluid	:	0.14M NaCl, 2mM K_2HPO_4, 0.4mM KH_2PO_4, 100 IU penicillin/ml, 97µg streptomycin/ml, 3% ethanol (pH not stated)
GLP	:	in compliance

The cutaneous absorption of Acid Red 92 was determined in a DMSO solution containing 1.67% of the test substance using pig skins *in vitro*. A dose of 100µl vehicle containing 1.67% of Acid Red 92 was applied on skin samples (1660 µg Acid Red 92/cm^2 pig skin) for 30 minutes and subsequently rinsed off with water and shampoo. After 72 hours, the amount of the test substance was determined in the receptor fluid, in the skin extracts (epidermis and upper dermis separated) and in the rinsing solution using HPLC analysis.

Results
The content of Acid Red 92 in all fractions in the receptor fluid was below 534 ng/cm^2 adding up all fractions. The content in all fractions was below the limit of quantification of 89 ng/cm^2 for single fraction. Considering the limit of quantification as upper limit, the amount of Acid Red 92 in the receptor fluid was < 0.534 µg/cm^2 (or < 0.032 % of the applied dose).
Correspondingly, the amount of < 0.534 µg/cm^2 was regarded as to have passed the skin barrier during the experimental period of 72 hours.
The concentrations of Acid Red 92 detected in the separated skin layers were 28.296 ± 5.326 µg/cm^2 (or 1.709 % ± 0322 %) in the epidermis, and 2.345 ± 0.476 µg/cm^2 (or 0.142 % ± 0.029 %) in the upper dermis. A total recovery of 93.7 % was calculated, including the amount of test substance in the rinsing solution (1520 µg/cm^2 or 91.821 %).

Conclusion
The laboratory concludes that, under the described test conditions, that correspond to realistic in use conditions, a dermal penetration rate of 0.534 µg/cm^2/72h was obtained.

However, adding the receptor fluid, epidermis and dermis values generates a percutaneous absorption value for Acid Red 92 of 31.175 ± 5.802 µg/cm² (with individual values of the 5 cells 24.442, 32.530, 28.576, 30.799, 39.531 µg/cm² respectively).

Ref.: 10

Percutaneous absorption, study 3

Guideline	:	/
Test system	:	Full thickness human skin (1000 µm), 5 samples (3 different donors). No justification for the use of full thickness skin is given
Contact time	:	30 minutes
Test substance	:	Acid Red 92, used at 1.67% (final) in a an oxidative formulation (composition not stated)
Control	:	composition not stated
Purity	:	94.0 - 99.5%
Batch no	:	not stated
Application	:	100 mg/cm²
Receptor fluid	:	0.14M NaCl, 2mM K_2HPO_4, 0.4mM KH_2PO_4, 100 IU penicillin/ml, 97µg streptomycin/ml, 3% ethanol (pH not stated)
GLP	:	in compliance

The cutaneous absorption of Acid Red 92 was determined in a representative hair dye formulation containing 1.67% of the test substance using human skin samples *in vitro*. A dose of 100 mg formulation/cm² was applied for 30 minutes and subsequently rinsed off with water and shampoo. After 72 hours, the amount of the test substance was determined in the receptor fluid and in the rinsing solution using HPLC analysis. Extractions of the skin extracts were not performed, since no effective methods for extraction of Acid Red 92 from skin were available.

Results
No Acid Red 92 was found in the receptor fluid and the majority of the substance was removed by the washing procedure after 30 minutes, namely 1314 ± 12 µg/cm² (corresponding to 78.8 ± 0.3% of the applied dose).

Conclusion
Since no measurements were performed in the different skin layers, this study does not generate any additional data concerning the penetration potential of the substance.

Ref.: 11

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Reverse Mutation Testing using Bacteria

Guideline	:	OECD 471 (1997)
Species/strain	:	*Salmonella typhimurium* (TA 1535; TA 1537; TA 98; TA 100; TA 102)
Test item	:	WR802177
Batch no.	:	AR92-020420
Purity	:	84.8 % (w/w) by NMR; stability: until 27.05.2007
Replicate	:	2 exp.
Dose levels	:	1, 10, 100, 1000, 5000 µg/plate
Metabolic act.	:	rat liver homogenate (S 9); induced by Aroclor 1254
Positive controls	:	according to OECD guideline
GLP	:	in compliance

Results
Toxicity: none.

Mutagenicity:	TA 98:	no increase in the no. mutants in all conditions;
	TA 100:	no increase in the no. mutants in all conditions; 5000 µg/plate toxic ± S 9;
	TA 1535:	a weak mutagenic activity in the presence of S9 of no relevance; toxic effect at the maximum dose with S9.
	TA 1537:	no increase in mutants in all conditions
	TA 102:	some increase with the dose in all conditions, but never reaching the double.

Conclusion
There is a very weak indication of induced mutagenicity. The study as such can be considered as negative. The test item is not mutagenic in this assay.

Ref.: 12

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

Guideline	:	OECD 476 (1997)
Specie/strain	:	Mouse lymphoma L5178 cells (Thymidine Kinase locus);
Test item	:	Fuchsia Red WR 802177.
Batch no.	:	AR92-020420.
Purity	:	99.5 area % (HPLC) 85 % (w/w) (NMR, calculated as sodium salt).
Replicate	:	Exp.1: ± S 9; Exp.2: -S 9;
Dose level	:	Exp.1: -S9: 5, 10, 20, 30, 40 µg/ml (4 hours) +S9: 5, 10, 20, 40, 60 µg/ml (4 hours) Exp.2: -S9: 19.5, 39, 78, 156 µg/ml (24 hours)
Metabolic act.	:	Phenobarbital/Naphthoflavone treated rat liver homogenate (S 9)
Positive contr.	:	MMS: without S9; 3-MC:with S9.
GLP	:	in compliance.

Results

Toxicity: in a preliminary experiment a relevant toxicity was observed at 39.1 µg/ml in all conditions at 4 h of treatment; in the 24 h of treatment a severe toxicity was observed at 312.5 µg/ml. The doses for the main experiments were chosen on the base of a preliminary toxicity experiment.

Mutagenicity:

A) positive controls
MMS (-S9): 4 h: 1st culture: small colonies: 164 (51); large colonies:114 (51); 2nd culture: small colonies: 225 (53),large colonies:157 (49).(in brackets the untreated control values)
24 h: small colonies: 1247 (61); large colonies: 346 (43)
3-MC (+S9):4 h.1st culture: small colonies: 260 (67); large colonies: 311 (67);
2nd culture: small colonies: 137 (44); large colonies: 121 (40).

The results obtained with the positive controls make acceptable the study.

B) Treated cells
A mutagenic effect observed in one culture was not repeated in the replicate culture.

Conclusion
Under the test conditions the test item is considered non mutagenic in this assay.

Ref.: 13

2.8.2 Mutagenicity/Genotoxicity *in vivo*

In vivo Mammalian Erythrocyte Micronucleus Test

Guideline	:	OECD 474 (1997)
Specie/strain	:	NMRI mice animal/sex/group
Test item	:	Fuchsia Red WR 802177
Batch no.	:	AR 92-029420
Purity	:	99.5 area % (HPLC); 88.5 % (w/w) (NMR, calculated as a sodium salt)
Dose level	:	25, 50, 100 mg/kg (24 h); 100 mg/kg (48 h)
Treatment	:	i.p. (10 ml/kg)
Positive control	:	CPA 40 mg/kg i.p.
GLP	:	in compliance

Results
Toxicity: 2 animals/sex were treated i.p. with the doses used in the final experiment and observed for 1,2-4,6,24,30,and 48 h. In a second experiment a dose of 200 mg/kg was i.p. administered to 4 animals (2M;2F).In a third experiment a dose of 150 mg/kg was i.p. administered to 4 animals (2m;2F).
150 and 200 mg/kg were toxic to the animals; the dose of 100 mg/kg induced some toxic effects. No justification of the i.p. treatment was presented.

Mutagenicity: the positive control (CPA) induced 2.030 % of MN in PCEs (0.08 % in the control animals: significance p 0.0040.
In the treated animals with 100 mg/kg a percentage of MN 0.150 (24h) and 0.110 (48h) was observed: these values, although higher than the control, had a $p > 0.34$.A reduction of PCE was observed, thus indicating a cytotoxic effect of the test item in the bone marrow cells.

Conclusion
Under the condition of this test, the test item has resulted a non mutagenic compound in the *in vivo* conditions, on mice.

Ref.: 14

2.9. Carcinogenicity

Oral administration, mice

Acid Red 92 was fed to (C57BL/6N x C3H/HeN) F1 mice. Groups of 49-64 males and 46-62 females received 0 (control), 0.1 and 0.4% Acid Red 92 in the diet starting at 6 week of age for a maximum of 90 weeks. Survival of mice was more than 85% at 64 weeks after start of Acid Red 92 administration. Both male and female mice given 0.1 and 0.4% Acid Red 92 weighed

significantly more than their respective controls. At 80 weeks, about 50% of surviving mice of all six groups were killed in order to ascertain the possible presence of any tumours and the remaining mice were subjected to autopsy at week 90. The number of pituitary tumours was increased in the female mice. Thus, while only one tumour (2%) was found in the control, 10 (20%) were found in the low dosed animals and 6 (12%) in the high dose animals. The percentage of animals with liver adenomas was not significantly affected by the treatment, while the number of adenoma per animal was increased among the dosed males. The authors do not comment on the pituitary tumours, and no data was given concerning variation in historical controls.

Ref.: A

Human studies

No data.

2.10. Special investigations

No data.

2.11. Safety evaluation

Not applicable

2.12. Conclusions

Physical and chemical characterisation
The information provided on the compound is largely incomplete and confusing regarding purity, impurities and physical constants (see general comments in the end of section 2.2.10.).
Toxicity
The NOAEL for systemic toxicity was set at 50 mg/kg bw/day, assuming that the signs of stomach irritation in the mid-dose group represent a local effect due to irritating potential of the test substance, and assuming that the mortalities were due to dosing errors.

Irritation & corrosivity
Acid Red 92 is neither considered a skin or eye irritant. However, the assessment of skin irritation with colorants as test substances is always difficult due to the staining of the test sites and thus masking of possible erythema.

Sensitisation
Acid Red 92 is not a skin sensitizer under experimental conditions stricter than use conditions. Therefore, Acid Red 92 does not pose a sensitizing risk to consumers when used as intended.

Teratogenicity
Acid Red 92 elicited slight maternal toxicity at 250 mg/kg bw/day but was not embryo-toxic or teratogenic at the doses tested. The NOAEL for maternal toxicity was considered to be 50 mg/kg bw/day.

Percutaneous absorption
Three in vitro percutaneous absorption studies were submitted (ref. 9-11). In all 3 studies:

- The concentration of the dye in the test solution was 1.67 % instead of the maximum requested concentration of 2 %.
- The exact composition of the hair dye formulation in which Acid Red 92 is incorporated, is not stated, neither is the composition of the control substance.
- The justification for the use of full thickness skin is not given. The laboratory mentions split thickness skin, but further description + the thickness of 1000 µm indicate the use of full thickness skin.
- Only 5 skin samples have been used.
- The dosage was extremely high, being 100 mg/cm² instead of up to 20 mg/cm² (SCCNFP Notes of Guidance). In such infinite dose percutaneous absorption studies, the obtained results expressed as a percentage, are of no value for any calculation.
- The choice of the receptor fluid, its pH and the solubility of Acid Red 92 in the receptor fluid are not documented.

In studies 1 and 3:
- Mixing with 2 volumes of oxidizing agent instead of 1.5 volumes proposed under functions and uses (section 2.10) is not justified; in the study 3 no measurements were performed in the different skin layers.

In study 2:
- The justification for the choice of the vehicle (DMSO) should be given.

For all the above mentioned reasons, all the 3 percutaneous absorption studies submitted (ref. 9-11) cannot be considered as acceptable.

Mutagenicity/Genotoxicity

Acid Red 92 has been tested in two *in vitro* assays for the induction of gene mutations and chromosome aberrations in bacterial cells and in mammalian cells. An *in vivo* study for the induction of numerical/structural chromosome aberrations has been performed on mice.
Under the conditions of the studies, the test item has been demonstrated to be devoid of the ability to induce gene mutations in bacterial and mammalian cells, to induce chromosome aberrations in mammalian cells *in vitro*, and numerical/structural chromosome aberrations *in vivo* in mice bone marrow.

Carcinogenicity

A long-term carcinogenicity study with mice showed an increased frequency of pituitary tumours among the dosed females. The frequency of the other tumours was not affected by the presence of Acid Red 92 in the diet. The study is not adequately reported.

2.13. References

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2. Acid Red 92: 28-Day oral toxicity (gavage) study in the Wistar rat; RCC Ltd, CH-4452 Itingen, Switzerland; Study number 844692; June 25, 2003
3. Acid Red 92: 13-Week oral toxicity (gavage) study in Wistar rats; RCC Ltd, CH-4452 Itingen, Switzerland; Study number 845834; 2003
4. Acid Red 92, 802177: Primary skin irritation study in rabbits (4-hour semi-occlusive application); RCC Ltd, CH-4414 Füllinsdorf, Switzerland; Study number 844721; September 17, 2002

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6. 802177 (Fuchsia Red) – Local lymph node assay; MDS Pharma Services, F-69210 Saint Germain sur l'Arbresle, France; Study number 762/021; December 2002
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9. Cutaneous absorption of WR802177 in formulation through pig skin in vitro; Cosmital SA, CH-1723 Marly 1, Switzerland; Study number KP 077; November 15, 2002
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11. Human skin penetration of hair dye Gardex Fuchsia Red from one formulation – in vitro assessment, An-eX analytical services Ltd., Study number C07/01/02; November 4, 2002
12. Assessment of the potential mutagenicity of WR802177 in the Ames reversion assay with Salmonella typhimurium; Cosmital SA, CH-1723 Marly 1, Switzerland; Study number AT 783; September 17, 2002
13. Cell mutation assay at the thymidine kinase locus ($TK^{+/-}$) in mouse lymphoma L5178Y cells with Fuchsia Red WR 802177; RCC – CCR GmbH, D-64380 Rossdorf, Germany; Study number 749702; November 19, 2002
14. Micronucleus assay in bone marrow cells of the mouse with Fuchsia Red WR 802177; RCC – CCR GmbH, D-64380 Rossdorf, Germany; Study number 749701; November 27, 2002
15. Stability in formulation; September 22, 2003. Wella AG; D-64274 Darmstadt

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A Ito A, Fujimoto N, Okamoto T, Ando Y, Watanabe H. Tumorigenicity study of phloxine (FR 104) in B6C3F1 mice. Fd Chem Toxic 32: 517-520, 1994.

3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the information submitted is inadequate to assess the safe use of the substance as a hair dye ingredient, either as an oxidative or semi-permanent.

Before any further consideration, the following information is required :

* complete physico-chemical characterisation of the test substances used;
* percutaneous absorption study in accordance with the SCCNFP Notes of Guidance;
* data on the genotoxicity/mutagenicity following the relevant SCCNFP-opinions and in accordance with the Notes of Guidance.

4. Other considerations

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5. Minority opinions

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