EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

Acid Red 52

COLIPA n° C177

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

The SCCP adopted this opinion at its 16th plenary of 24 June 2008

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 Products (SCCP), 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 Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCP

Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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1. BACKGROUND

Submission I for Acid Red 52 with the chemical name Hydrogen-3,6-bis(diethylamino)-9-(2,4-disulphonatophenyl)xanthylium (sodium salt) was received in September 2003.

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted by written procedure on 23 April 2004 an opinion (SCCNFP/0803/04) that, "*the use of Acid Red 52 as a hair colouring agent in semipermanent hair dye formulas at a maximum concentration of 0.6 % in the finished cosmetic product does not pose a risk to the health of the consumer*."

Acid Red 52 is regulated as a cosmetic colorant under CI 45100 in Annex IV of the Directive.

Submission II was submitted in December 2006.

2. TERMS OF REFERENCE

1. *Does SCCP consider Acid Red 52 safe for consumers when used as an ingredient in oxidative hair dye products with a maximum concentration of 1.5% on the scalp, taken into account the scientific data provided?*

2. *Does the SCCP recommend any further restrictions with regard to the use of Acid Red 52 in any hair dye formulations?*

3. OPINION

A complete safety dossier for semi-permanent hair dye formulations was submitted in September 2003. Based on this data, the SCCP concluded that Acid Red 52 as a hair colouring agent in semi-permanent hair dye formulations at maximum concentration of 0.6% in the finished cosmetic product does not pose a risk to the health of the consumer (opinion n° SCCNFP/0803/04).

There is, however, a need for further evaluation of the dye under oxidative conditions with a maximal on-head concentration of 1.5%.

Acid Red 52 is a direct dye which is stable with H_2O_2. In order to consider different penetration behaviours which might be influenced by H_2O_2, the penetration study was repeated under realistic conditions as described in this submission.

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

Taken from SCCNFP/0803/04

3.1.1.1. Primary name and/or INCI name

Acid Red 52 (INCI name)

3.1.1.2. Chemical names

Hydrogen-3,6-bis(diethylamino)-9-(2,4-disulphonatophenyl)xanthylium (sodium salt)

3.1.1.3. Trade names and abbreviations

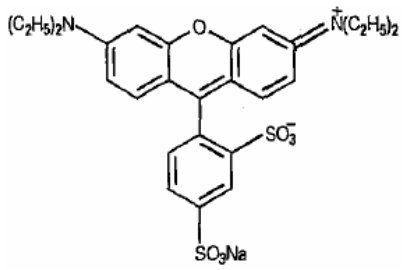
Acid leather Red KB
Acid Red XB
Acid Rhodamine B
Aizen Food Red n° 106
Amacid Rhodamine B
Amido Rhodamine B
Brilliant Acid Rhodamine B
Brilliant Superlan Rhodamine B
Brilliant Superlan Rhodamine
Erio Acid Red XB
Fenazo Pink XXB
Food Red 106
Kiton Rhodamine B
Lissamine Rhodamine
Pontacyl Brilliant Pink 2B Red 106
Red n° 106
Rose Covasol W 4002
Solar Rhodamine B
Sulforhodamine B
Xylene Red B

CI 45100
COLIPA n° C177

3.1.1.4. CAS / EINECS number

CAS: 3520-42-1
ELINCS: 222-529-8

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{27}H_{29}N_2NaO_7S_2$

3.1.2. Physical form

Green/brown to pink powder, depending on purification method

3.1.3. Molecular weight

Molecular weight: 580.7 g/mol

3.1.4. Purity, composition and substance codes

Chemical characterisation by FTIR, NMR, MS and UV-Vis

Batch comparison

	Lot 18882	Lot B2015	Lot B3101
Dye content, HPLC peak area at 258 nm and 552 nm	≥ 99%	> 90%	> 90%
Volatile matters at 105°C	3.9 %	2.3 %	2.9 %
Lead	≤ 10	≤ 10	≤ 10
Copper (ppm)	≤ 5	≤ 5	≤5
Zinc (ppm)	≤ 8	≤ 8	≤ 8
Chromium (ppm)	≤ 8	≤ 8	<≤ 8
Cadmium (ppm)	≤ 1	≤ 1	≤ 1
Barium (HCl 0.07N) (ppm)	≤ 10	≤ 10	≤ 10
Arsenic (ppm)	≤ 3	≤ 3	≤ 3
Mercury (ppm)	≤ 1	≤ 1	≤ 1
Antimony (ppm)	≤ 10	≤ 10	≤ 10
Absorbance maxima (nm)	565	565	565
Maximum absorbance of the 5 mg/l solution in water	0.802	0.802	0.758

Sulfate Ash content: < 4%
Other organic colorants (unidentified 0.5-1.3%, HPLC peak area)
Remaining up to 100%: inorganic salts

3.1.5. Impurities / accompanying contaminants

See 3.1.4

3.1.6. Solubility

up to 7.5% in water
up to 3% in saline
up to 1% in DMSO
up to 2 % in formulation

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 1.3 at pH 7.15 (EU method A8)

3.1.8. Additional physical and chemical specifications

Melting point:	/
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
UV_Vis spectrum (200-800 nm)	λmax 565 nm

3.1.9. Homogeneity and Stability

Acid Red 52 is stable under normal laboratory conditions.
The dye is stable under oxidative conditions: 98.4-109.4% recovery, 60 min after mixing the oxidative dye formulation.
The dye is stable for one day in PBS buffer (receptor fluid used for dermal absorption) and in HPLC eluent mixture; and also after freezing and thawing the dye in this mixture.
10, 30 and 100 mg/ml solution of the dye in 1% aqueous CMC were stable at room temperature for 4 hours study period (variation from nominal concentration: -5 to + 4%).
Aqueous solutions of the dye were stable for 2 hours at room temperature (20°C) and 7 days at 4°C.
The test solutions of the dye used in the toxicity studies were shown to be homogenous (variation 2-8% in the top, middle and bottom of the stock solutions).

General Comments to physico-chemical characterisation

- Documentation for the chemical characterisation is provided only of one batch (18882).
- Absolute content of Acid Red 52 in the test materials used in various studies is not reported.
- Water solubility of Acid Red 52 has not been determined by the EU Method
- The stability of Acid Red 52 in the marketed products is not known

3.2. Function and uses

Acid Red 52, a non-reactive dye, is used up to a concentration of 1.5% on-head in oxidative hair dye formulations.
Acid Red 52 is used up to 0.6 % in non-oxidative hair dye-formulation.

Acid Red 52 is also used as cosmetic colorant according to Annex VI of the Cosmetic Directive.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCNFP/0803/04

LD50-oral-rat > 1000 mg/kg bw.

No details are available on study design and purity of the test material. There is no lethality in long term study observed even at high concentration.
Despite the shortcomings related to this study, there appears to be no need for repetition of the oral LD50 study with the rat.

Ref.: 1, 2

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/0803/04

Guideline:	/
Species/strain:	Albino-Himalaya rabbits
Group size:	6 animals
Observation period:	3 days
Test substance:	Acid Red 52
Purity:	/
Batch:	/
Dose level:	0.5 g (24h contact under occlusion)
GLP:	not in compliance

The primary skin irritation potential of Acid Red 52 (quality not specified) was investigated by topical occlusive application of 0.5 g to one intact and one additionally scarified flank of each of six Albino-Himalaya rabbits. The duration of the treatment was 24 hours. The scoring of skin reactions was performed immediately, 48 hours and 73 hours after removal of the occlusive patch.

Results
The test item did not elicit any skin reactions at the application site of any animal (all scores = 0).

Conclusion
The skin irritation study is old (1976) and performed on a substance of which the exact purity is unknown. Although this study is scientifically not valid, it seems unnecessary to perform additional animal studies, especially viewing the low percentage of the compound in its final formulation (0.6%).

Ref.: 3

3.3.2.2. Mucous membrane irritation

Taken from SCCNFP/0803/04

Guideline:	/
Species/strain:	Albino-Himalaya rabbits
Group size:	6 animals
Observation period:	3 days
Test substance:	Acid Red 52
Purity:	/
Batch:	/
Dose level:	0.1 g
GLP:	not in compliance

The primary eye irritation potential of Acid Red 52 was investigated by instillation of 0.1 g into the conjunctival sac of the left eye of each of six Albino-Himalaya rabbits. The right eye remained untreated and served as reference control. Scoring of irritation was performed 1, 7, 24, 48 and 72 hours after application with a magnifying glass. After 24 hours all eyes were rinsed with physiological saline.

Results
Because of colour interference, an exact judgement was not possible in the area of the cornea (1 and 7 hours after post application). The 24h-48h-72h mean scores were as follows:

$S_{cornea\ opacity}$	=	0.00
S_{iris}	=	0.00
$S_{conjunctiva\ redness}$	=	0.67
$S_{conjunctiva\ chemosis}$	=	0.06

Therefore, the test substance is considered as non-irritating to the rabbit eye.

Conclusion
The eye irritation study is old (1976) and performed on a substance of which the exact purity is unknown. Although this study is scientifically not valid, it seems unnecessary to perform additional animal studies, especially viewing the low percentage of the compound in its final formulation.

Ref.: 3

3.3.3. Skin sensitisation

Taken from SCCNFP/0803/04

Guideline:	OECD 406 (1992)
Species/strain:	Himalayan spotted albino guinea pig
Group size:	10 females in test group, 5 females in control group
Observation period:	25 days
Test substance:	Acid Red 52
Batch:	18882
Purity:	≥ 99% (HPLC, peak area)

Dose levels:	intradermal induction:	5% in 1% CMC solution
epidermal induction:	50% in 1% CMC solution	
epidermal challenge:	10% in 1% CMC solution	
(preliminary screening study available)		
GLP:	in compliance	

The test group consisted of 10 female guinea pigs and one control group of five female guinea pigs. Test item (purity specified about 80%; Lot.18882) 25%, w/w was solved in 1% CMC (prepared with bi-distilled water). A pre-test was performed in order to assure an optimum technical application procedure. As a result of this, the main study was performed as follows:

Induction: Intradermal induction of sensitization (day 1) in the test group was performed with Freund´s Complete Adjuvant (FCA) and physiological saline (1:1), test item at 5% in 1% CMC, 5% dilution of the test item in 1% CMC in a 1:1 mixture with FCA / physiological saline. The epidermal induction of sensitisation (day 8) was conducted under occlusion with the test item at 50% in 1% CMC for 48 hours.

Challenge: The challenge was performed at day 22 by application of the test item at 10% in 1% CMC under occlusive patch for 24 h at a different part of the skin.

Cutaneous reactions were evaluated at 24 and 48 hours removal of the dressings.

Results
After challenge no skin reactions were observed.

Conclusion
The test compound is regarded as non-sensitising.

Ref.: 4

3.3.4. Dermal / percutaneous absorption

Taken from SCCNFP/0803/04

Guideline:	OECD 428 (1995)
Test system:	split thickness pig skin (400 µm), 6 samples
Contact time:	30 minutes under occlusion (donor chamber covered with parafilm)
Test substance:	Exp. I: Acid Red 52, 5 mg/ml in saline pH 3.0 Exp. II: Hair Colour Gel CI L 366I, containing 0.5% Acid Red 52 (pH 2.9 - 3.1)
Control:	caffeine, tested every 3 months, results available
Batch:	18882
Purity:	≥ 99% (HPLC, peak area)
Application:	1 ml (density considered □□1 kg/l)
Receptor fluid:	Saline solution, pH 3.0
GLP:	in compliance

Porcine ear obtained from the slaughter house immediately after slaughter and before steam cleaning were used for this experiment. The outer ear region was washed, carefully shaved and the skin was removed by dissection. Thickness of the dissected skin was approximately 400 µm. The surface of the skin which was in contact with the test substance during permeation-assay was 1.01 cm². Two experiments were performed:

Experiment I:	5 mg/ml pure dye (pH 3; Lot. 18882) was dissolved in saline (clear specification known)
Experiment II:	A viscous ready to use standard formulation was directly applied to the skin:

Standard formulation:
50-74 % Water
15-25 % Propylene Carbonate
5-10 % Alcohol
1- 5 % Lactic Acid
1- 5 % Hydroxypropylated polysaccharide
1- 5% Dimethicone Copolyol
0.1 – 1 % Fragrance
< 0.1 % Sodium Hydroxide
0.5% Acid Red 52 (C.I. 45100; Lot. 18882)

The skin was mounted in glass flow-through diffusion chamber with diameter of 1.135 cm. Each donor chamber was filled with 1 ml of the test item dissolved in saline, pH 3.0 or one gram of the formulation and covered with parafilm. Since the pH of the representative hair dye formulation is 3.0, this pH was used in both experiments.
Saline, pH 3.0 was pumped through the chambers with a flow rate of 1-2 ml/hour and chamber. Buffer solution of the acceptor chamber was collected in plastic vials which were replaced according to the sampling times and stored at – 20 °C. The whole test system was set up in an incubator adjusted to 32 °C. After 30 min of incubation, test item was removed from skin with 10% aqueous shampoo solution. Following the washing procedure the donor chamber were filled with 1 ml of saline pH 3.0. The collecting vials were changed after 0, 0.5, 1, 2, 4, 6, 8 and 24 hours. After skin extraction the item bound at the stratum corneum was quantified.

Results
No measurable permeation through the skin occurred at any time point within the time frame of both experiments. The lowest detection limit under the conditions reported is 150 ng/ml. The amount/cm² of the test item found to have crossed the skin barrier (measurements in the receptor fluid) is 10.1 µg/cm² in the first and 8.3 µg/cm² in the second experiment.
However, the amount of penetrated test item found in the receptor fluid plus that found in the skin extract are considered as penetrated respectively absorbed. Since the stratum corneum was not separated from the epidermal and dermal compartment, the amounts found in the skin extract
are to be added to those in the receptor solution. The individual amounts found in the skin extract were:

Experiment I: 0.27 - 0.28 - 0.75 - 0.93 - 1.81 - 3.65 µg/cm²
Experiment II: 1.38 - 2.01 - 2.03 - 3.10 - 3.39 - 9.02 µg/cm²

The mean recovery of the test item was 111 % in the first and 97.3 % in the second experiment.

Conclusion
Many shortcomings can be formulated with regard to this study:
- Dosage should be expressed as µg/cm² or µl/cm² throughout the whole report.
- As indicated by the sponsor, no separate measurements have been performed on stratum corneum, epidermis and dermis.
- No data on the solubility of the test substance in the receptor fluid are given. Moreover, the receptor fluid is used as a vehicle in one of the experiments.
- The company states that the amount of Acid Red 52 found in the skin extract should be added to the amount found in the acceptor chamber. However, when giving their final figures, only the acceptor chamber values are taken into consideration.
- There is an unacceptably large variability in the skin extract measurements (0.27 - 3.65 µg/cm² in Exp. I and 1.38 - 9.02 µg/cm² in Exp. II), which makes is impossible to make a correct assessment of the percutaneous absorption of Acid Red 52.

- The test has been performed with an 0.5% Acid Red 52 formulation, while the requested maximum authorized concentration is 0.6%.

For all the above mentioned reasons, the percutaneous absorption study cannot be accepted.

Ref.: 12

New study, submission II

Guideline:	OECD 428 (2004)
Tissue:	porcine ear skin, 400 µm thickness, 12 donors
Group size:	2 x 6 chambers (2 excluded from analysis)
Diffusion cells:	static chambers, 1.0 cm² application area
Skin integrity:	conductivity measurement (< 900 µS)
Test substance:	Acid Red 52
Batch:	18882
Purity:	≥ 99% (HPLC, peak area)
Test item:	C1 E 2005166/2 (3% Acid Red 52)
Oxidative agent:	developer lotion Topchic 6% 20 vol.
Test formulation:	test item + oxidative agent (1:1)
Doses:	20 µl/cm² corresponding to 153.4 µg/cm² Acid Red 52
Receptor fluid:	phosphate buffered saline (PBS)
Solubility receptor fluid:	/
Stability:	stable up to 48h in PBS and eluent mixture
Method of Analysis:	HPLC
GLP:	in compliance
Study period:	27 – 31 March 2006

Acid Red 52 was assessed for its potential for dermal absorption on porcine skin under oxidative conditions.

Two independent experiments were performed under static conditions with 6 diffusion cells per experiment. For the analysis of the dermal absorption of Acid Red 52, one chamber was excluded from the analysis due to a technical defect and one chamber was excluded since the recovery was below 85%. The 10 remaining chambers met the acceptance criteria and were therefore were used for the analysis of Acid Red 52.

The study was performed on freshly dermatomed pig skin samples that were used on the day of slaughter and mounted on diffusion cells between donor and receptor chambers. The conductivity across the skin samples of each cell was determined before treatment and after the last sampling as a measure of skin integrity. The blank samples (at 0 hours) were collected immediately after filling the donor chambers, but before application of the test item.

In each experiment, 6 replicates of the formulation were analysed. 20 µL of the formulation was applied to each sample for 30 minutes and then washed off using 2 x 1 mL deionised water. 3 x 1 mL 10% shampoo solution, and 2 x 1 mL deionised water. The penetration was determined after 24 hours under non-occluded static conditions. Phosphate buffered saline (PBS) was used as the receptor fluid.

After 24 hours, the donor chambers were filled with 1 mL receptor solution to re-analyse the conductivity. The stratum corneum was separated by tape stripping from the remaining dermis and epidermis. The tape strips (2 x 5 strips per sample) were pooled as two batches and extracted for analysis. The samples were analysed by HPLC for the presence of Acid Red 52.

Result
Under the reported conditions, the dermal absorption of Acid Red 52 was 0.33 ± 0.39 µg/cm² or 0.21 ± 0.26% (mean value of 10 diffusion cells (10 donors)).

Table 1: summary of test results

	Experiment 1					
Chamber	**1**	**2**	**3**	**4**	**5**	**6**
Dermal absorption (µg/cm²)	0.144	0.186	0.116	0.116	0.294	0.075
Dermal absorption (%)	0.089	0.110	0.069	0.069	0.184	0.045
	Experiment 2					
Chamber	**1**	**2**	**3**	**4**	**5**	**6**
Dermal absorption (µg/cm²)	1.396	0.262	0.378	0.188	17.389	0.168
Dermal absorption (%)	0.925	0.185	0.247	0.119	11.315	0.117
Mean dermal absorption, experiment 1 and 2 ± SD	**0.33 ± 0.39 µg/cm² or 0.21 ± 0.26%**					

Results of the shaded chambers were not used for the calculation of the dermal absorption since either the recovery was less than 85% or the integrity of the chamber was not guaranteed.

Table 2: individual test results

	Experiment 1 (µg/cm²)					
Chamber	**1**	**2**	**3**	**4**	**5**	**6**
Strip Sol [1]	0.809	0.850	1.045	2.223	1.978	1.740
Skin extract [2]	0.128	0.101	0.103	0.095	0.250	0.059
Sum receptor fluid [3]	0.016	0.085	0.013	0.021	0.044	0.016
Dermal absorption [4]	**0.144 (0.089%)**	**0.186 (0.110%)**	**0.116 (0.069%)**	**0.116 (0.069%)**	**0.294 (0.184%)**	**0.075 (0.045%)**
	Experiment 2 (µg/cm²)					
Chamber	**1**	**2**	**3**	**4**	**5**	**6**
Strip Sol [1]	1.270	0.430	1.040	0.736	6.542	0.677
Skin extract [2]	1.396	0.262	0.318	0.138	17.274	0.063
Sum receptor fluid [3]	0.00	0.00	0.060	0.050	0.115	0.105
Dermal absorption [4]	**1.396 (0.925%)**	**0.262 (0.185%)**	**0.378 (0.247%)**	**0.188 (0.119%)**	**17.389 (11.315%)**	**0.168 (0.117%)**

[1] Stripping solution: concentration of Acid Red 52 in the extract solution of the strips (adsorption)
[2] Skin extract: concentration of Acid Red 52 in the extract solution of the remaining skin (absorption)
[3] Total amount of Acid Red 62 found in the receptor solution (penetration)
[4] Dermal absorption: the sum of the amount of Acid Red 52 found in the receptor fluids and in the skin extracts

Ref.: 15

Comment
Because of the high standard deviation, the mean value + 2 standard deviations will be used for the calculation of the Margin of Safety, namely 1.11 µg/cm² (0.33 + 2 x 0.39).

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (14 days) oral / dermal / inhalation toxicity

Taken from SCCNFP/0803/04

Guideline:	OECD 407 (1981)
Species/strain:	Wistar rat
Group size:	10 animals (5 males & 5 females) / dose level
Observation period:	14 days (no recovery group)
Test substance:	Acid Red 52
Batch:	18882
Purity:	≥ 99% (HPLC, peak area)
Dose levels:	0, 100, 300, 1000 mg/kg bw/day
GLP:	not in compliance

Four groups of five male and five female Wistar rats received Acid Red 52 daily by gavage at doses of 0, 100, 300 and 1000 mg/kg bw/day for 14 days. The test material (purity specified about 80%, Lot. 18882) was homogenized in bi-distilled water containing 1% Carboxymethylcellulose sodium salt (CMC). Clinical signs, food consumption and body weights were recorded periodically during pre-test, and treatment period. All animals were killed, necropsied and examined post mortem.

Results
No death occurred during the 14-day treatment period.
In all treated animals, violet faeces were observed until the end of the treatment period. This was considered to be a typical passive effect resulting from oral administration of the dye and is not considered to be a sign of toxicity. The absolute and relative kidney weights of males treated with 1000 mg/kg bw/day was lower than those of control animals. This finding was considered to be incidental.

Conclusion
On the basis of the results obtained in the 14-day dose range finding study the following dose levels were selected for the 90 day subchronic toxicity study: 0, 100, 300, 1000 mg/kg bw/day.

Ref.: 5

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

Taken from SCCNFP/0803/04

Guideline:	OECD 408 (1998)
Species/strain:	Wistar rat
Group size:	20 animals (10 males & 10 females) / dose level
Observation period:	91 days (no recovery group)
Test substance:	Acid Red 52
Batch:	18882
Purity:	≥ 99% (HPLC, peak area)
Dose levels:	0 - 100 - 300 - 1000 mg/kg bw/day
GLP:	in compliance

Three groups of ten male and ten female Wistar rats received the test substance, Acid Red 52 daily by gavage at doses of 0, 100, 300 or 1000 mg/kg bw/day for 13 weeks. The test material (purity specified about 80%, Lot. 18882) was homogenized in bi-distilled water containing 1% CMC. Animals were observed twice daily for mortality/morbidity and once

daily for clinical abnormalities. Individual animal weights were recorded weekly. Body weight and food consumption were recorded weekly. Ophthalmologic evaluations on control and high-dose animals were performed at the end of the study. Haematology, clinical chemistry and urinalysis evaluations were performed once during week 13. At the end of the treatment period, all animals were killed and grossly examined. Selected organs were weighted. All animals were submitted to a complete macroscopic examination.

Results
No deaths occurred during the study. No test article related changes in body weight, food consumption, locomotor activity, grip strength, haematology and urinalysis were noted at all dose levels. Test item-related findings were:

100 mg/kg bw/day:	violet, respective black faeces, red discoloration of the mucosal surface of the stomach and /or intestine
300 mg/kg bw/day:	violet, respective black faeces, decreased uric acid levels in females, red discoloration of the mucosal surface of the stomach and /or intestine
1000 mg/kg bw/day:	slightly increased locomotor activities, violet, respective black faeces, discoloration of tail and paws, red discoloration of the mucosal surface of the stomach and /or intestine, decreased uric acid levels in both sexes, decreased fibrinogen levels, increased ß-globulin levels in males, decreased billirubin and increased phospholipid levels in females (none of the clinical biochemical aberrations correlated to any histological findings, wherefore they were regarded to be of no toxicological significance; they are considered to be metabolic adaptations to the test article)

Conclusion
Based on the results described above, the No-Observable-Adverse-Effect-Level (NOAEL) of Acid Red 52 (CI 45100) is considered to be 1000 mg/kg bw/day when administered by gavage over a period of 13 weeks.

Ref.: 6

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Taken from SCCNFP/0803/04

Bacterial Reverse Mutation Assay

Guideline:	OECD 471 (1997)
Species/Strain:	*S. typhimurium* (TA1535, TA1537, TA 98, TA 100); *E. coli* (WP2uvrA)
Test item:	Acid Red 52 (CI 45100)
Batch:	18882
Purity:	≥ 99% (HPLC, peak area)
Replicate:	2 experiments
Dose:	Exp.1: 33; 100; 333; 1000; 2500; 5000 µg/plate Exp.2: 250; 500; 1666.7; 1250; 2500, 5000 µg/plate
Metabolic activ.:	Phenobarbital/Naphthoflavone induced rat liver homogenate (S9)
Positive controls:	Sodium Azide (TA 1535, TA 100) 4-NOPD (TA 1537,TA 98)

MMS (WP2 uvr A)
2AA (+S 9: all strains)
GLP: in compliance

Results
Toxicity: 8 concentrations tested: some reduction of the revertant colonies background was observed at the doses 2500 and 5000 µg/plate.
Mutagenicity: No increase of the revertant colonies in the treated plates of the experiments was observed in all conditions.

Conclusion
The test item did not induce gene mutations in bacterial cells in all tested groups and under all conditions.

Ref.: 9

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

Guideline:	OECD 476 (1997)
Species/strain:	Mouse lymphoma L 5178 Y cells; Thymidine kinase locus
Test item:	Acid Red 52 (CI 45100)
Batch:	18882
Purity:	≥ 99% (HPLC, peak area)
Replicate:	1 exp. + S9-mix (4 h); 2 experiments - S9-mix (4 h; 24 h): 2 cultures/exp.
Dose levels:	with and without S9-mix: 156.3; 312.5; 625; 1250; 5000 µg/ml
Metabolic activ.:	Phenobarbital/Naphthoflavone induced rat liver homogenate (S9-mix)
Positive controls:	MMS (-S9-mix); 3-MC (+S9-mix)
GLP:	in compliance

Results
Toxicity: A reduction of the relative cell suspension growth, depending on the dose, with and without metabolic activation was observed.
Mutagenicity: The positive controls MMS (without S9-mix) induced small and large mutant colonies significantly higher than the untreated control in the two experiments on both cultures.
The positive control 3-MC (+ S9-mix) induced small and large mutant colonies significantly higher than the untreated control in the two cultures of the exp.1.
On the base of these results, the study is considered adequate.

No increase in the mutation frequencies of both small and large mutant colonies in the presence and the absence of S9 was observed in the replicate experiments.

Conclusion
The test item does not induce gene mutations and structural chromosome aberrations in the in vitro mammalian cell line.

Ref.: 10

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Taken from SCCNFP/0803/04

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

Guideline:	OECD 474 (1997)
Species/strain:	NMRI mice (5 animals/sex/group)
Test item:	Acid Red 52 (CI 45100)

Batch:	B 3101
Purity:	> 90% (HPLC, peak area)
Dose levels:	24 h: 500; 1000; 2000 mg/kg 48 h: 2000 mg/kg
Treatment type:	oral (gavage); one administration of 10 ml/kg
Positive control:	Cyclophosphamide (CPA): 40 mg/kg, oral
GLP:	in compliance.

Results
Toxicity: A dose of 2000 mg/kg of the test item was administered orally once and the animals were observed during 48 hours, when no toxic effects were reported.
Mutagenicity: The positive control, CPA, induced 1.085 % of MN/PCE per animal. The untreated animals presented 0.075 % of MN/PCE per animals.
The three doses of the test item in the 24 hours treatment induced respectively with the doses 0.04; 0.05; 0.05 % of MN/PCE per animal; in the 48 h of treatment a dose of 2000 mg/kg induced 0.03 MN/PCE per animal.

There was no sign of cytotoxicity induced by the test item in the bone marrow cells; however, analysis of the serum revealed a presence of the test item after 1 hour, 8 to 15 times higher than the detection limit.

Ref.: 11

3.3.7. Carcinogenicity

Taken from SCCNFP/0803/04

Male and female Fischer 344/DuCrj rats (groups of 50 males and 50 females) received Acid Red 52 mixed in the basal diet at doses of 0 (control), 2.5, and 5.0 % for 2 years. The treatment started when the animals were 6 weeks of age. The mean cumulative intake of Acid Red 52 over the 106 experimental weeks was computed to be 356 and 738 g/rat for males at the 0.5 and 5.0% levels, respectively, while females consumed on the average 252 and 523 g/rat in the low and high dose group, respectively. Body and organ weights haematology, urinalysis, and histopathological evaluations revealed no evidence of adverse effects associated with the compound relative to the untreated controls. The spectrum, incidence, and histology of tumours developing in both treated and control animals were consistent with spontaneous incidences reported in this strain of rat. The authors conclude that the study indicate that Acid Red 52 is not carcinogenic to F344 rats after 2 years of dietary administration at maximum level of 5.0% in the basal diet.

Ref.: A

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Taken from SCCNFP/0803/04

Dose-range finding prenatal development toxicity study

Guideline:	/
Species/strain:	Wistar rat
Group size:	5 females / dose level
Observation period:	20 days

Test substance:	Acid Red 52
Batch:	18882
Purity:	≥ 99% (HPLC, peak area)
Dose levels:	0, 100, 300, 1000 mg/kg bw/day
GLP:	not in compliance

Three groups of five mated female rats were administered Acid Red 52 by gavage at doses of 100, 300 or 1000 mg/kg bw/day from day 6 through day 17 post coitum. An additional group of five mated rats was administered the vehicle (bi-distilled water containing 1% carboxymethylcellulose sodium salt) and served as a control group. On day 21 post coitum, the animals were killed and examined macroscopically. Foetuses were removed by Caesarean section.

Results
No death occurred during the course of the study. Violet discoloured faeces, urine and bedding material were noted from day 7 post coitum till day 19 post coitum in all treatment groups. The relevant maternal reproductive data (mean number of implantations and live foetuses) were similar in all groups. No external abnormalities were noted in any of the foetuses. The sex ratios were similar in all groups.

Conclusion
Based on the result of this study, dose levels of 100, 300 and 1000 mg/kg bw were used in the main study for effects on embryo-foetal development.

Ref.: 7

Teratogenicity study

Guideline:	OECD 414 (1981)
Species/strain:	Wistar rat
Group size:	22 females / dose level
Observation period:	20 days
Test substance:	Acid Red 52
Batch:	18882
Purity:	≥ 99% (HPLC, peak area)
Dose levels:	0 - 100 - 300 - 1000 mg/kg bw/day
GLP:	in compliance

Three groups of 22 pregnant rats received Acid Red 52 by gavage at doses of 100, 300 or 1000 mg/kg bw/day from day 6 through day 17 post coitum. A fourth group of 22 pregnant rats received the vehicle only (bi-distilled water containing 1% carboxymethylcellulose sodium salt) and served as a control group.
Animals were checked twice daily for mortality/morbidity, and once daily for clinical signs. Food consumption and body weight were recorded at designated intervals during pregnancy. On day 21 post coitum, the animals were killed and examined macroscopically. Foetuses were removed by Caesarean section.

Results
All animals survived until Caesarean section and with the exception of violet discoloured urine, faeces and bedding material observed in all dosage groups, no reaction to treatment or clinical signs were observed in any female. Food consumption and body weighty development were not affected by the test substance administration. No abnormal macroscopically findings were noted during necropsy.
The differences amongst the relevant reproduction data (post-implantation loss, number of implantations and foetuses) of the vehicle control group and the dose groups gave no indication of test article related effects.

The mean body weights of foetuses, the ratio of male and female foetuses and the results of external, visceral and skeletal examinations of foetuses gave no indication of effects caused by administration of the test article.

Conclusion
Based on the results described above, 1000 mg/kg bw/day of Acid Red 52 (C.I. 45100) is considered to be the No-Observable-Adverse-Effect-Level (NOAEL) for the maternal organism and the NOEL (no observed effect level) for the foetal organism.

Ref.: 8

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

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

(Acid Red 52)
(Direct / semi-permanent)

Maximum absorption through the skin	**A (µg/cm^2)**	**=**	**1.11 µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**700 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.777 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.013 mg/kg bw**
No observed adverse effect level (13-week, oral, rat)	**NOAEL**	**=**	**1000 mg/kg bw**
Margin of Safety	**NOAEL / SED**	**=**	**76923**

3.3.14. Discussion

Physico-chemical properties
Acid Red 52 is used up to 0.6 % in non-oxidative hair dye-formulation and up to an on-head concentration of 1.5% in oxidative hair dye formulations. Acid Red 52 was shown to be stable under oxidative conditions.
Absolute content of Acid Red 52 in the test materials used in various studies is not reported. Documentation for the chemical characterisation is provided only of one batch (18882). Water solubility of Acid Red 52 has not been determined by the EU Method. The stability of Acid Red 52 in the marketed products is not known
Acid Red 52 is also used as cosmetic colorant according to Annex VI of the Cosmetic Directive, but the present evaluation concerns only to the use of Acid Red 52 in oxidative hair dye formulations

Toxicity
The NOAEL of Acid Red 52 (CI 45100) was set at 1000 mg/kg bw/day when administered by gavage over a period of 13 weeks.

Skin/eye irritation and sensitisation
The skin and eye irritation study are old (1976) and performed on a substance of which the exact purity is unknown. Although this study is scientifically not valid, it seems unnecessary to perform additional animal studies, especially viewing the low percentage of the compound in its final formulation (0.6%).
Acid Red 52 is regarded as non-sensitising.

Percutaneous absorption (oxidative conditions)
Because of the high standard deviation, the mean value + 2 standard deviations will be used for the calculation of the Margin of Safety, namely 1.11 µg/cm² (0.33 + 2 x 0.39).
In a previous opinion (SCCNFP/0803/040), the safety calculation for the use of Acid Red 52 under non-oxidative conditions had been based on the assumption of 100% absorption due to an inadequate absorption study.

Mutagenicity/genotoxicity
Acid Red 52 has been tested for its potential mutagenicity/genotoxicity in two *in vitro* assays (gene mutations in bacteria and in mammalian cells) and in an *in vivo* assay (mammalian erythrocyte micronucleus test). It may be concluded that Acid Red 52 neither induces gene mutations in bacterial and mammalian cells, nor chromosome aberrations in mammalian cells *in vitro* and *in vivo.*

Carcinogenicity
A long-term carcinogenicity study with rats did not indicate any cancer hazard.

4. CONCLUSION

The SCCP is of the opinion that the use of Acid Red 52 as an ingredient in oxidative hair dye formulations, at a maximum concentration of 1.5% on the head or in non-oxidative hair dye formulations at a maximum concentration of 0.6% on the head, does not pose any risk to the health of the consumer.

Its use as a colorant was not evaluated.

5. MINORITY OPINION

Not applicable

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