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

CONCERNING

ACID RED 52

COLIPA n° C177

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 52 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 52 is listed as CI 45100 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 4: colouring agents allowed exclusively in cosmetic products intended to come into contact only briefly with the skin.

2.1.1. Primary name

Acid Red 52 (INCI)

2.1.2. Chemical names

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

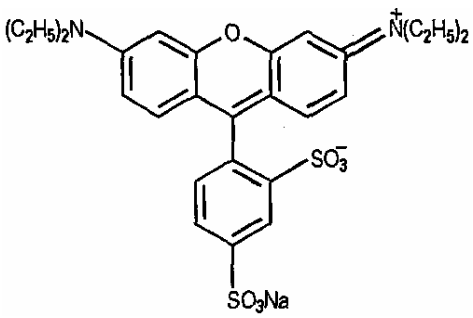
2.1.3. Trade names and abbreviations

COLIPA n°	:	C177	
Other names	:	Food Red 106	Rose Covasol W 4002
		Amido Rhodamine B	Erio Acid Red XB
		Amacid Rhodamine B	Acid leather Red KB
		Xylene Red B	Aizen Food Red n° 106
		Sulforhodamine B	Acid Red XB
		Solar Rhodamine B	Acid Rhodamine B
		Red n° 106	Pontacyl Brilliant Pink
		Red 106	Brilliant Acid Rhodamine B
		Kiton Rhodamine B	Brilliant Superlan Rhodamine B
		Lissamine Rhodamine	Brilliant Superlan Rhodamine 2B
		Fenazo Pink XXB	

2.1.4. CAS / EINECS / COLOR INDEX number

CAS	:	3520-42-1
EINECS	:	222-529-8
Colour index	:	CI 45100

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{27}H_{29}N_2NaO_7S_2$
Mol weight : 580.7

2.1.7. Purity, composition and substance codes

Substance code : /
Batches used : /

Purity : > 90 %
Loss on drying : 5 % max (volatile matter 105°C)
Water content : /
Ash content : /

Impurities

Lead	:	max. 10 ppm
Copper	:	max. 100 ppm
Zinc	:	max. 100 ppm
Chromium	:	max. 100 ppm
Cadmium	:	max. 1 ppm
Barium (HCl 0,07N)	:	max. 50 ppm
Arsenic	:	max. 3 ppm
Mercury	:	max. 1 ppm
Antimony	:	max. 10 ppm

Solvent Residues : /

The purity of the tested batch (18882) is indicated to be 80 ± 3%, while the purity of that same batch is indicated as 99% in the company's summary.

2.1.8. Physical properties

Appearance : Green/brown to pink powder, depending on purification method
Melting point : /
Boiling point : /
Density : /
Rel. vap. dens. : /
Vapour Press. : /
Log Pow : 1.3 (pH 7.15)
pKa : /

2.1.9. Solubility

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

2.1.10 Stability

No data

General comments on analytical and physico-chemical characterisation

* The information provided on the compound is incomplete.

2.2. Function and uses

Acid Red 52 is intended to be used in non-oxidative hair dye formulations at a maximum concentration of 0.6%.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

LD_{50}-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 LD_{50} study with the rat.

Ref.: 1, 2

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

Guideline	:	OECD 407 (1981)
Species/strain	:	Wistar rat
Group size	:	10 animals (5 males & 5 females) / dose level
Observ. Period	:	14 days (no recovery group)
Test substance	:	Acid Red 52
Batch no	:	18882
Purity	:	80% (± 3%)
Dose levels	:	0, 100, 300, 1000 mg/kg bw/day

GLP statement : /

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 pretest, 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 a dye stuff 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

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	:	Wistar rat
Group size	:	20 animals (10 males & 10 females) / dose level
Observ. Period	:	91 days (no recovery group)
Test substance	:	Acid Red 52
Batch no	:	18882
Purity	:	80% (± 3%)
Dose levels	:	0 - 100 - 300 - 1000 mg/kg bw/day
GLP statement	:	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. Hematology, 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, hematology 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

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 : /
Species/strain : Albino-Himalaya rabbits
Group size : 6 animals

Observ. Period	:	3 days
Test substance	:	Acid Red 52
Purity	:	/
Batch no	:	/
Dose level	:	0.5 g (24h contact under occlusion)
GLP	:	/

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

2.4.2. Irritation (mucous membranes)

Guideline	:	/
Species/strain	:	Albino-Himalaya rabbits
Group size	:	6 animals
Observ. Period	:	3 days
Test substance	:	Acid Red 52
Purity	:	/
Batch no	:	/
Dose level	:	0.1 g
GLP	:	/

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 :

$<<S_{\text{cornea opacity}}>>$ = 0.00
$<<S_{\text{iris}}>>$ = 0.00
$<<S_{\text{conjunctiva redness}}>>$ = 0.67
$<<S_{\text{conjunctiva chemosis}}>>$ = 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 (0.6%).

Ref.: 3

2.5. Sensitisation

Guideline	:	OECD 406 (1992)
Species/strain	:	Himalayan spotted albino guinea pig
Group size	:	10 females in test group, 5 females in control group
Observ. Period	:	25 days
Test substance	:	Acid Red 52
Purity	:	80% (± 3%)
Batch no	:	18882
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 pretest 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

2.6. Teratogenicity

Dose-range finding prenatal development toxicity study

Guideline	:	/
Species/strain	:	Wistar rat
Group size	:	5 females / dose level
Observ. Period	:	20 days
Test substance	:	Acid Red 52
Batch no	:	18882
Purity	:	80% (± 3%)
Dose levels	:	0, 100, 300, 1000 mg/kg bw/day
GLP statement	:	/

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% carboxymethyl cellulose 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 body weight 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
Observ. Period	:	20 days
Test substance	:	Acid Red 52
Batch no	:	18882
Purity	:	80% (± 3%)
Dose levels	:	0 - 100 - 300 - 1000 mg/kg bw/day
GLP statement	:	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 macroscopical 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

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Percutaneous absorption

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
Purity	:	80% (± 3%)
Batch no	:	18882
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 ^{o}C. The whole test system was set up in an incubator adjusted to 32 ^{o}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

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Reverse Mutation Testing using Bacteria

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	:	about 80% (i.e.± 3 %)
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	:	About 80 % (i.e. ± 3 %)
Replicate	:	1 exp. + S 9 (4 h); 2 experiments - S 9 (4 h; 24 h): 2 cultures/exp.

Dose levels	:	± S 9: 156.3; 312.5; 625; 1250; 5000 µg/ml
Metabolic activ.	:	Phenobarbital/Naphthoflavone induced rat liver homogenate (S 9)
Positive controls	:	MMS (- 9); 3-MC (+ S 9)
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 (- S 9) induced small and large mutant colonies significantly higher than the untreated control in the two experiments on both cultures.
The positive control 3 - MC (+ S 9) 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 S 9 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

2.8.2 Mutagenicity/Genotoxicity *in vivo*

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 no.	:	B 3101
Purity	:	About 80% (the certificate is not related to this specific batch: May 2002 and not January 2002.
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

2.9. Carcinogenicity

Oral administration, rat

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

Human studies

No data

2.10. Special investigations

No data

2.11. Safety evaluation

CALCULATION OF THE MARGIN OF SAFETY

The percutaneous absorption study results cannot be used to calculate the MoS. Therefore, the worst case of 100% will be used in the calculation. The Notes of Guidance (SCCNFP/0690/03) indicate a weekly use of 35 ml for a semi-permanent hair dye, with a retention factor of 0.1.

Absolute worst case calculation (assuming a daily use of 35 ml and a relative density of approximately 1.0 for the hair dye formulation):

Maximum absorption through the skin	A (%)	=	100%
Typical body weight of human		=	60 kg
Daily exposure to hair dye formulation		=	35g/day
Retention Factor		=	0.1
Concentration of dye in the formulation		=	0.6%
Systemic exposure dose (SED)		=	0.35 mg/kg/day
No observed effect level (mg/kg)	NOAEL	=	1000 mg/kg

Margin of Safety	**NOAEL / SED = 2857**

2.12. Conclusions

Physico-chemical properties
The information provided on the compound is incomplete.

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
The percutaneous absorption study results cannot be used to calculate the MoS. Therefore, the worst case of 100% will be used in the calculation.

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 nor 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.

2.13. References

1. Smart PL. A review of the toxicity of twelve fluorescent dyes used for water tracing, NSS Bulletin, 46, 21-33, 1984
2. RTECS, Acid Red 52, 2002.
3. Haut und Schleimhautverträglichkeit von Amidorhodamin B (konz.) an Kaninchen; Bericht 408/76; Hoechst Aktiengesellschaft Pharma Forschung Toxikologie, 25.10.1976
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3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the use of Acid Red 52 as a hair colouring agent in semi-permanent 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.

4. Other considerations

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5. Minority opinions