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

CONCERNING

ACID YELLOW 1

COLIPA n° B1

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 Yellow 1 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 Yellow 1 is listed as CI 10316 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 2: colouring agents allowed in all cosmetic products except those intended to be applied in the vicinity of the eyes, in particular eye make-up and eye make-up remover.

2.1.1. Primary name

Acid Yellow 1 (INCI)

2.1.2. Chemical names

Disodium 5,7-dinitro-8-oxido-2-naphthalene sulfonate
2-Naphthalenesulfonic acid,8-hydroxy-5,7-dinitro-, disodium salt

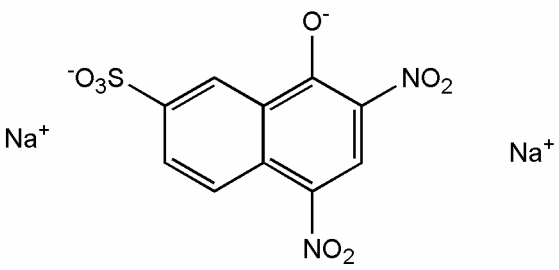
2.1.3. Trade names and abbreviations

Trade name	:	Ext. D&C Yellow Nº 7; Japan Yellow 403
COLIPA n°	:	B 1
Other names	:	Citronin A
		Flavianic acid sodium salt
		Naphthol Yellow S
		Japan Yellow 403
		Ext D&C Yellow nº 7

2.1.4. CAS / EINECS / Colour Index number

CAS n°	:	846-70-8
EINECS n°	:	221-690-2
Colour index	:	CI 10316

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{10}H_6N_2O_8SNa_2$
Mol weight : 358.19

2.1.7. Purity, composition and substance codes

Batches used: a certified colour Ext D&C Yellow 7 batch 14067, Lot AG 6738 was used in the toxicological studies. All analytical data are related to batch B3943.

Purity

Titre as determined by NMR	:	86.3% (quantitative)
Water content	:	7% (w/w)
Sulphated ash content	:	38.9%
Salt content (as Na_2SO_4)	:	4.3%
Heavy metals	:	< 124 ppm

Relative chromatographic purity (HPLC - UV/VIS peak area method)
99.6% (qualitative) (253 nm)
99.8 % (430 nm)

Potential impurities
Reagents and intermediate reaction products

1-Naphthol	:	< 0.2%
2,4-Dinitro-1-naphthol	:	< 0.03%
1-Naphthol-2,4,7-trisulfonic acid	:	/
1-Naphthol-4,7-disulfonic acid	:	/
1-Naphthol-2,7-disulfonic	:	/

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

2.1.8. Physical properties

Appearance	:	Yellow powder
Melting point	:	>300 ºC
Boiling point	:	716.8 ºC (calculated) *
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	4.49E -17 hPa (calculated) *
Log P_{ow}	:	1.218 ± 1.235 (calculated) *
pKa	:	/

* See General Comments below

2.1.9. Solubility

6.6% soluble in water (pH 6.2), 4.1% soluble in acetone/water (1:1), > 10% soluble in DMSO.

2.1.10 Stability

The test substance on storage in dryness and darkness is stable more than 24 months. In aqueous solutions containing 1% CMC is stable at 20ºC for at least seven days. Using the same conditions, in acetone/water 1:1, the recovery is: 99.0-100.3% and in DMSO (10%), the recovery is 97.6-99.9%.

General comments on analytical and physico-chemical characterisation

* The information provided on the compound is largely incomplete and confusing, not conforming to the SCCNFP Notes of Guidance. Analytical data are reported for a batch different from the batch used in toxicological studies and for which the purity is reported either as 89% or 97.5% (HPLC). There is confusion between absolute content of the dye and its chromatographic purity determined by the relative peak-area HPLC method.

* The following by-products have not been determined:
  1-Naphthol-2,4,7-trisulfonic acid
  1-Naphthol-4,7-disulfonic acid
  1-Naphthol-2,7-disulfonic

* Some inorganic impurities (sodium salts) are present (4.3%).

* The content of chloride and sulphate are pending of quantification.

* Some 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).

* The pKa values of the ionisable groups are not reported.

* No justification is provided for the wide range (1.218 ± 1.235) of the reported calculated value of log P_{ow}. It is not known if the calculation has taken into consideration a pH value related to physiological conditions and to the conditions of the percutaneous absorption studies. Since log P_{ow} is known to strongly depend from the pH, the reported values are useless and confusing.

* The stability of the dye in a prototype formulation is not given.

2.2. Function and uses

Acid Yellow 1 is intended for use in semi-permanent hair dye formulations as a direct dye at a maximum concentration of 0.2% and in oxidative hair dyes at a maximum final concentration of 1.0 % after mixing with 1:1.5 of hydrogen peroxide preparation.

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	:	/
Species/strain	:	Rat, HanBrl: WIST (SPF)
Group size	:	5 males + 5 females
Test substance	:	Ext. D&C Yellow 7
Purity	:	Colour content 89 %
Batch no	:	14067
Dose levels	:	0, 100, 300 and 1000 mg/kg bw/day, by gavage
Exposure period	:	14 days
GLP	:	/

All animals were killed after 14 days of treatment. *Post mortem* examination of liver, kidneys, spleen, adrenals, heart and any gross lesions were conducted in all animals. Tissues were fixed in neutral phosphate buffered 4% formaldehyde solution and retained for possible further histopathological examination.

Results

All other animals survived until scheduled necropsy, except for one female of the high dose group that was sacrificed for ethical reasons on test day 12 as it was emaciated with ruffled fur, convulsing remaining in a ventral position. Hunched posture was noted in 3 females of the high dose group and persisted until the end of the study period.

No dose-related effects were noted in food consumption when compared with the control group. In females dosed with 300 or 1000 mg/kg bw/day, body weight gain was slightly reduced after the 14-day treatment period. Bodyweight of all other animals was comparable with controls.

Discoloured (yellow-orange) faeces were seen in all animals dosed with the test substance. This was considered to be a typical passive effect resulting from oral administration of dyestuff and not considered a sign of toxicity.

The organ weights and the organ/body weight ratios of the spleen were statistically significantly increased in all animals treated with 1000 mg/kg bw/day. In all males treated with 300mg/kg bw/day this finding was also observed, but without statistical significance. This finding is considered to be dose-related. The organ/body weight ratio of the kidneys was statistically significantly increased in females treated with 1000 mg/kg bw/day. No dose-related macroscopic findings were observed.

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)
Size	:	10 males and 10 females per dose
Test item	:	Acid Yellow 1
Batch no.	:	B3943 (Goldmann)
Purity	:	97.5 % (HPLC)
Dose	:	0, 30, 100 and 300 mg/kg body weight/day
Vehicle	:	1% aqueous carboxymethylcellulose
Route	:	oral, by gavage
GLP	:	/

The safety dossier indicates a GLP but there is no data to support this. The study report was not signed by the Study director. The pathology data is a draft.

On the basis of the results from the 14-day range-finding study, dose levels of 0, 30, 100 and 300 mg/kg bw/day were proposed for this 90-day subchronic toxicity study.
Clinical signs, outside cage observations, food consumption and body weights were recorded periodically during pre-test, the treatment period. Ophthalmoscopic examinations were performed at pre-test and at the end of the treatment. A functional observational battery including locomotor activity and grip strength were performed during week 4.
Post mortem examinations were done on all animals. Histology of organs and tissues from the control and high dose groups, as well as on gross lesions from all animals in the study were performed. In addition, the kidneys (females only) as well as the intestine, liver and spleen (both sexes) were examined from all animals of the intermediate group.

Results
All animals survived until scheduled necropsy. Oral dosing resulted in no adverse dose-related clinical signs during daily observation.
Slightly red soft faeces were found in all treated animals from week one onwards. The urine was also tinted deep yellow in all treated animals. The pH of the urine at all doses in males and in top dose in females increased but was within historical controls. These were considered to be passive effects of the dyestuff.
Slight non-specific alopecia, skin scaliness and bilateral mioisis in almost all dose groups. These effects were considered incidental.
Significantly reduced mean absolute body weights were seen in females treated with 300 mg/kg bw/day from treatment week 9 to 13, and significantly reduced mean body weight gain were seen in females of the same dose group from treatment week 8 to 13 and in week five.
There was an increase in mean absolute reticulocyte count after 13 weeks in all animals dosed at 100 mg/kg/day and 300 mg/kg/day($p<0.01$) compared with controls. A decrease in haemoglobin in all animals at 300 mg/kg/day ($p<0.01$) was also noted. The mean corpuscular volume increased in females dosed at 100 mg/kg/day ($p<0.05$) and in all animals at 300

mg/kg/day($p<0.01$) compared with controls. The mean absolute neutrophils were increased in all animals at 300 mg/kg/day($p<0.05$). All these were considered to be treatment-related since they were outside the range of the historical controls.
Under the conditions of this study, dose-related lesions were seen in the intestine, spleen and liver of animals from both sexes at 100 and 300 mg/kg bw/day and in the kidneys in females at 300 mg/kg bw/day. Lesions, described as gross nodules, were found in the caecum in two males at 100 mg/kg bw/day and in all animals at 300 mg/kg bw/day. There were indications of primary toxicity, ulceration and /or inflammation in the mucosa/submucosa (caecum) in animals at 300 mg/kg bw/day. There was occasional mucosal hyperplasia. The study authors thought these were more likely to be caused by increased faecal passage time through this section rather than receptor related interactions. In other intestinal segments of some animals at this dose, there was regenerative diffuse mucosal hyperplasia of minor severity.
Significantly increased spleen weights, spleen to body weight ratios and spleen to brain weight ratios were noted in males dosed at 100 mg/kg bw/day or in both sexes at 300 mg/kg bw/day compared with controls. Increased spleen weight seemed to be related to an increase in extramedullary haemopoiesis in both sexes and increased haemosidirin deposition of females. Consecutive bleeding in the spleen may be the reason for adaptive changes recorded in the spleen of animals at 100 and 300 mg/kg bw/day.
Minimal centrilobular hepatocellular hypertrophy was seen in 5 males and one female at 300 mg/kg bw/day. This hypertrophy was ambiguous, since it was followed by the statement 'but was not accompanied by further effects, as there was Kupffer cell proliferation, increased apoptosis, necrosis, fibrosis etc'.

In the kidneys of 8 females at 300 mg/kg bw/day, a moderate to severe diffuse basophilia in the corticomedullary junction, accompanied by tubular epithelial hypertrophy and by karyomegaly was observed.
Based on the results of the study, 30 mg/kg bw/day of Acid Yellow 1 was established as the no observed adverse effect level (NOAEL).

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)

No data

2.4.2. Irritation (mucous membranes)

Guideline	:	/
Species/strain	:	Human keratinocytes from cell line HaCaT
Test item	:	Ext. D&C Yellow No. 7
Batch no.	:	B3943 (Goldmann)
Purity	:	97.5% (HPLC)
Dose	:	0 to 10000 µg/ml
GLP	:	/

A modified Neutral Red Uptake (NRU) assay to assess the eye irritancy potential by measuring the cytotoxicity was performed according to the Standard Operating Procedure developed for COLIPA International Validation Study on alternatives to the Draize Rabbit Eye Irritation Test. The modifications were the use of human keratinocytes cell line (HaCaT) in a serum-free culture medium. An in-house classification system was used instead of the COLIPA prediction model. Monolayers of human keratinocytes (HaCaT) were exposed in 96 well microtiter plates to various concentrations of the test item, for 24 hours. Cell viability was measured by neutral red uptake. The concentration causing 50 % reduction in neutral red uptake in treated cells compared with untreated control cells (NRU-50) was determined.

Results
In a range-finding study, NRU-50 value of the test substance between 10000 and 3162 µg/ml were determined.
Based on these results, 8 doses between 681 and 10000µg/ml were selected for two defined NRU assays. The median NRU-50 value of both independent assays with the test item was 6916 µg/ml.. The NRU-50 values of sodium lauryl sulphate, used as a reference substance, were within the historical range of the laboratory.

Conclusion
The NRU assay provides information on the relative cytotoxicity, enabling comparison of similar formulations or chemicals with similar structure. The test results provide information to assess the irritation potential of new test substances in comparison with other substances or benchmarks. Therefore, the NRU test system has only a limited value as a replacement of the established "Acute Dermal Irritation/Corrosion" test (OECD 404).
The study authors concluded that the test substance is non-irritant, according to the in-house NRU classification system for the assessment of eye irritation potential based on the NRU-50, but qualified this ‘where the NRU assay is performed as part of an *in vitro* test battery’.

Ref.: 4

2.5. Sensitisation

Local Lymph Node Assay

Guidelines	:	OECD 429 (2000)
Species/strain	:	Mouse: CBA/J
Size	:	5 females per dose and vehicle
Test item	:	Ext. D&C Yellow 7, (Acid Yellow 1)
Batch no.	:	B3943 (Goldmann)
Purity	:	97.5 % (HPLC)
Dose	:	0.3, 1, 3, 10 % in DMSO

0.3, 1, 3, 4.1 % in acetone/water (1:1) mixed with olive oil (4:1)

GLP : /

The safety dossier indicates GLP but there is no signed statement to support this. This is Draft 1 of the study report and as such was not signed by the Study director.

Acid Yellow 1 was tested at different concentrations in two vehicles. On days 0, 1 and 2, the animals received 25 µl of the test item formulation, positive control or vehicle on the dorsal surface of each pinna.

Morbidity/mortality checks were performed generally 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 via ^{3}H-thymidine incorporation.

Results
In DMSO: The test item induced a negative response, as there was less than a 3-fold increase in isotope incorporation in the draining auricular lymph nodes relative to the vehicle. The mean stimulation indices were 0.9, 1.2, 0.9 and 1.1 at the concentrations of 0.3 %, 1 %, 3 % and 10 %, respectively.

In acetone/water (1:1) mixed with olive oil (4:1): The test item induced a negative response, as there was less than a 3-fold increase in isotope incorporation in the draining auricular lymph nodes relative to the vehicle. The mean stimulation indices were 1.2, 1.6, 1.0 and 1.0 at the concentrations of 0.3 %, 1 %, 3 % and 4.1 %, respectively.

The positive control, 1%. p-phenylenediamine (PPD), produced a mean stimulation index of 6.5. This was more than the required 3-fold increase in isotope incorporation in the draining auricular lymph nodes relative to the vehicle.

The test item is not a skin sensitizer under the defined experimental conditions in the two vehicles tested.

Ref.: 5

Magnusson and Kligman Maximisation Test

Guidelines	:	OECD 406 (1992)
Species/strain	:	Ibm: GOHI; SPF-quality guinea pigs
Size	:	15 females (10 test group and 5 control group)
Test item	:	Ext. D&C Yellow 7 (Acid Yellow 1)
Batch no.	:	14067
Purity	:	certified total colour content: 89 %
Dose	:	10 % solution (challenge)
GLP	:	in compliance

In order to assess the cutaneous allergenic potential of Acid Yellow 1, the Maximization-Test was performed in 15 (10 test and 5 control) female albino guinea pigs, in accordance with OECD Guideline 406 and the Directive 96/54/EEC, B.6.

The intradermal induction of sensitisation in the test group was performed in the nuchal region with a 5 % dilution of the test item in 1 % CMC (carboxymethylcellulose) and in an emulsion of Freund's Complete Adjuvant (FCA) / physiological saline. The epidermal induction of sensitisation was conducted for 48 hours under occlusion with the test item at 50% in 1 % CMC one week after the intradermal induction. The animals of the control group were intradermally induced with 1% CMC and FCA / physiological saline and epidermally induced with 1 % CMC under occlusion.
Two weeks after epidermal induction the control and test animals were challenged by epidermal application of the test item at 10% in 1% CMC and 1% CMC alone under occlusive dressing.
Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing.

Results
No toxic symptoms were evident in the guinea pigs of the control or test group. No deaths occurred.
All test animals showed discrete/patchy to moderate/confluent erythema at the 24- and 48-hour reading after the challenge treatment with Acid Yellow 1 at 10% (w/w) in 1% CMC. No skin effect was observed in the control group.
Based on the results, the test item has to be classified as a skin sensitizer.

Ref.: 6

2.6. Teratogenicity

Prenatal developmental toxicity (teratogenicity) study

Guidelines	:	OECD 414 (2001)
Species/strain	:	Rat WIST HanBRL: WIST (SPF)
Size	:	96 mated females 24 per group"
Test item	:	Acid Yellow 1,
Batch no.	:	B3943 Goldmann
Purity	:	97.5 %
Stability	:	max 4h @ room temperature
Dose	:	0, 50, 150 and 450 mg/kg bw/day from day 6 - 20 of gestation
Vehicle	:	1 % aqueous carboxymethylcellulose
Route	:	oral, by gavage
GLP	:	in compliance

Acid Yellow 1 was tested for its embryotoxic, foetotoxic and teratogenic potential in rats. Dams were killed on day 21 p.c., just prior to expected delivery, and foetuses were removed by Caesarean section for examination.

Results
Maternal Data:
One female, dosed450 mg/kg bw/day, was found dead on the day of scheduled necropsy (gestation day 21.). Prior to death, this female displayed ruffled fur and hunched posture for three days. At necropsy, dark brown spleen was seen. At this dose level, all females had red coloured faeces, ruffled fur and / or hunched posture.
Food consumption was reduced by 22 % in the 450 mg/kg bw/day over the entire treatment period and by 8% in the150mg/kg bw/day from gestation days 9 and 12 compared with the control group.
Body weight gain was significantly reduced during the treatment period in group dosed at 450 mg/kg bw/day,. The overall weight gain during gestation was reduced by 49 %. The resulting

body weights were significantly reduced from day 10 post-coitum. At necropsy, mean body weight was significantly reduced, uterus weight was slightly reduced (reduced foetal weights) and weight loss (corrected body weight from day post-coitum) was observed.
At necropsy, all females at 450 mg/kg bw/day had dark brown spleen discoloration. No reason for this discolouration nor spleen weights were provided.
At 150 mg/kg bw/day, all females had slightly red coloured faeces which correlated with the colour of the test substance. It was not considered to be an adverse effect.

Reproduction data:
Mean number of implantation sites, pre- and post-implantation losses and mean number of foetuses per litter and group were not affected by treatment. There were no dead foetuses.

Foetal data:
The sex ratios were similar in all groups.
At 450 mg/kg bw/day, mean foetal body weights were significantly reduced (17 %) compared with the control group. There were minor skeletal variations consisting of incomplete ossification of sternebrae, metatarsal -1, talus and phalanges. This incomplete ossification was associated with the overall reduced development as a result of maternal toxicity. The study authors considered it was a minor developmental delay.
Conclusion
On the basis of these results, the study authors derived a no observable adverse effect level (NOAEL) for maternal toxicity at 50 mg/kg bw/day and for foetuses 150 mg/kg bw/day. There was no indication of teratogenic potential.

Ref.: 7

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Percutaneous Absorption *in vitro*

Guideline	:	/
Tissue	:	Ear pig skin obtained by dissection
Method	:	Flow-through Franz diffusion cells
Test substance 1	:	Acid Yellow 1 dissolved in saline at pH 3.0 (5 mg/ ml)
Test substance 2	:	Representative hair dye formulation (pH 3.0) containing 0.5% Acid Yellow 1
Batch No	:	AG 6738 (dye) ; 14067 (formulation)
Dose Test item 1	:	1 ml of solution; 5mg/cm^2 of the dye active principle
Dose Test item 2	:	1.2 g of formulation; 6 mg/cm^2 of the dye active principle
Receptor fluid	:	Saline (pH 3.0)
Replicate cells	:	6 cells
Analyt. method	:	HPLC (Detection at 450 nm) Quantitation limit: 150 ng/ml
GLP	:	in compliance

The skin penetration of Acid Yellow 1 was evaluated in a flow-through Franz diffusion cell system using pig skin. The thickness of the dissected skin was about 100 µm in the first and between 540-780 µm in the second experiment. The integrity of the skin was checked by conductivity and no loss of barrier properties of the skin was detected. The solubility of the dye in the receptor fluid was not provided.

Doses of 1ml or 1.2 g were applied on skin samples (6 mg /cm^2 and 5 mg /cm^2 for representative formulation and saline solution, respectively) for 30 min. Then, the skin surface was rinsed off with shampoo and water and left unoccluded for the entire 24 hour exposure period. The donor chamber was filled with 1 ml of saline (pH 3.0) and the collecting vials of the receptor chamber were changed after 0.5, 1, 2, 4, 6, 8, and 24 hours. At the end of the experiment, the dye content was determined in the receptor fluid, in the skin extracts and in the rinsing solutions using HPLC analysis. Caffeine was used as a positive control.

Results
Under the present experimental conditions, a total recovery of the dye of 106.4% and 104.3% was obtained for the saline solution and the representative formulation, respectively.
The content of Acid Yellow 1 in the receptor fluid was in all cases below the limit of detection of 150 ng/ml (150 ng/cm^2). Amounts of 5.7µg/cm^2 (0.13% of the applied dose) and 5.5 µg/cm^2 (0.09% of the applied dose) were regarded as to have passed the skin barrier after 24 hour exposure for the saline solution and the representative formulation, respectively.
The concentrations of Acid Yellow 1 detected in the skin extracts were 0.63 µg/cm^2 (0.013% of the applied dose) and 13 µg/cm^2 (0.22% of the applied dose) for the saline solution and for the formulation, respectively. Considering both tests, a global percutaneous absorption of 18.8 µg/cm^2 is reported.

Comments
- The solubility of the dye in the receptor fluid is not provided.
- The procedure to obtain the skin extracts is not explained.
- The test should be performed in the presence of the oxidative reagent.
- The pH of the receptor fluid (3.0) is not appropriate.

As a conclusion, the methodology used can be considered inappropriate according to the Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients, adopted by the SCCNFP.

Ref.: 8

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, TA98, TA100); *E. coli* (WP2 uvrA)
Test items	:	Ext D&C Yellow 7 (CI 10316)
Batch No.	:	14067; Lot AG6738
Purity	:	certified total colour content: 89% (+volatile matter: 70%). Stored at room temperature, light and humidity protected. Exp. Date: July 2001
Replicate	:	1st experiment: plate incorporation. 2nd experiment pre-incubation
Doses	:	33; 100; 333; 1000; 2500; 5000 µg/plate
Metabolic Act.	:	S9 liver microsomal activation from Rats treated with 80 mg/kg bw Phenobarbital and β-Naphthoflavone
Positive controls	:	According to OECD/471 Guideline
GLP	:	in compliance

Results
Toxicity : the preliminary experiments were conducted to evaluate the toxicity. A reduction on cell viability was noted at the maximum dose in TA1535, TA1537, TA98, TA100, WP2 uvrA. In the first experiment (plate incorporation) and in the second experiment (pre-incubation) there was no indication of induced reverse mutations on all strains and on all conditions.

Conclusion
The test item is considered non mutagenic in this assay.

Ref.: 9

***In vitro* Mammalian cell gene mutation test**

Guideline	:	OECD/476 (July 1997)
Species/Strains	:	Mouse lymphoma L5178Y cells (Forward mutations at thymidine kinase (TK +/-) locus
Test item	:	Ext D&C Yellow 7 (C.I 10316)
Batch No	:	14067; Lot AG6738
Purity	:	Certified total colour content: 89% (+volatile matter: 7%). Stored at room temperature, light and humidity protected. Expiration date: July 2001
Replicate	:	1st exp : with and without metabolic activation. 2nd exp: without metabolic activation
Doses	:	without S9: 250, 500, 100, 2000, 4000 µg/ml (2 experiments). With S9: 250, 500, 100, 2000, 4000 µg/ml
Metabolic Act.	:	S9 liver microsomal activation from rats treated with 80 mg/kg bw Phenobarbital and β-Naphthoflavone
Positive controls	:	(with metabolic activation) 3-methylcholanthrene (3-MC); (without metabolic activation) methyl methane sulphonate (MMS)
GLP	:	in compliance

Results
Toxicity : by using the same mutagenicity test conditions, the experiment was performed on one culture, by treating the cells for 4 and 24h (-S9) and for 4h (+S9). No toxicity was observed at any of the doses tested.

Mutagenicity : small and large colonies were counted in all treated plates. First experiment (4h treatment: ± S9) one culture. The Mutation Frequency (MF) of MMS (-S9) positive control was $197x10^6$ cells (control: 87) for small colonies and $105x10^6$ cells (control: 24) for large colonies. The MF of 3-MC (+S9) positive control was 247 per 10^6 cells (control: 79) for small colonies and $110x10^6$ cells (control: 35) for large colonies.
In the second culture produced almost the same frequencies. The second experiment, performed in the absence of S9 for 24h of treatment, MMS produced almost the same data for both cultures. These data indicate that the results of the treated cells with the test item are acceptable, because the two positive controls behaved as expected, thus allowing the evaluation of potential activity induced by the test item either gene mutations and chromosome aberrations. However, no historical data are reported for the two positive controls in relation to small and large colonies. The test substance did not indicate any increase in mutation frequency of either small or large colonies compared with the control cultures.

The absence of an induced effect in the treated cell populations was repeated in the second culture for each treatment condition, in the absence and in the presence of a metabolic activation system. No osmolality was observed.

Conclusion
The test item is considered non mutagenic and non clastogenic.

Ref.: 10

2.8.2 Mutagenicity/Genotoxicity *in vivo*

In vivo Mammalian Erythrocyte Micronucleus Test

Guideline	:	OECD/474 (1997)
Species/Strain	:	NMRI Mice
Group size	:	5 males /5 females / group dosed
Doses	:	12.5-25-50 mg/Kg
Test item	:	ACID YELLOW 1/23122/CI 10310
Batch No.	:	B 3943 (Goldmann)
Purity	:	99.6% area (HPLC 254 nm) 99.8% area (HPLC 430 nm) Stability not indicated by the Sponsor. Expiration date: October 2004
Doses	:	females 2x500; 2x1000; 2x2000 mg/kg bw males 2x450; 2x900; 2x18000 mg/kg bw
Positive control	:	CPA; 40 mg/kg bw orally, once;
Negative control	:	Deionised water
Administration	:	Oral, twice at 24h interval
Sacrifice time	:	24 after the second treatment
GLP	:	in compliance

Results
Toxicity in a first experiment (2M; 2F) a dose of 2000 mg/kg was administered orally twice: after the second experiment, beside some non relevant effects (activity reduction, eyelid closure, ruffled fur, urine colour) no death was observed.

Micronucleus
In the main experiment some toxic symptoms were observed of the type already indicated.
The percentage of MN in PCEs for the positive control was 1.38% in males and 0.61% in females; the respective untreated values were 0.05% on males and 0.03%.
At the dose of 2x1800 mg/kg bw, the value observed for the MN in the males was 0.13. This value represented a p of 0.05. All other animals did not show any significant increase in the percentage of MN. No reduction of the PCE per 2000 erythrocytes was observed, thus indicating that the test item was not cytotoxic.

Conclusion
The study was performed with a sacrifice time of 24h after the second treatment. A significant increase (p = 5%) of the frequency of MN in the male was observed.

The study authors concluded that the test substance did not induce micronuclei in mouse bone marrow. The significant increase of micronulei in males cannot be discounted, questioning this interpretation.

Ref.: 11

2.9. Carcinogenicity

Animal studies

Skin painting studies in Swiss Webster mice were carried out with a series of 11 coal-tar-derived colours including Acid Yellow 1. The treatment groups contained 50 males and 50 females and the control groups contained 100 males and 100 females. Mice were painted once weekly in an area that precluded oral exposure with 0.1 ml containing 1.0% Acid Yellow 1 to a depilated 6 cm^2 area. Survival, body weight, and palpable growth were followed for a 18 month period. Microscopic examination which initially involved 50% of the treated animals was extended to include all tumours and grossly abnormal tissues and organs. There was no significant difference between treatment and control groups.

Ref.: 12

Human studies

No data

2.10. Special investigations

No data

2.11. Safety evaluation

Not applicable

2.12. Conclusions

The chemical characterisation of Acid Yellow 1 is incomplete.

A NOAEL of 30 mg/kg bw/day was derived. A NOAEL for maternal toxicity of 50 mg/kg bw/day, and for foetal toxicity of 150 mg/kg bw/day were set. There was no indication of teratogenic potential.

The data on irritation is incomplete. No data were provided for skin irritation. The assessment of eye irritation potential was based solely on the NRU-50, which is currently not validated.

The sensitization potential is equivocal. Acid Yellow 1 showed a sensitising potential in the skin sensitisation test with guinea pigs, but not in the local lymph node assay.

The percutaneous absorption methods used were not considered appropriate (according to the Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients, adopted by the SCCNFP). The solubility of the dye in the receptor fluid was not provided. The pH of the

receptor fluid (3.0) is not appropriate. The method to obtain the skin extracts was not explained. The test should have also been performed in the presence of the oxidative reagent.

Acid Yellow 1 was tested for the potential induction of gene mutations on bacterial cells (OECD/471) and of the gene mutation/clastogenic effect in mouse lymphoma cells (OECD/476). In these two studies the same sample of the test item was tested. The results indicate that Acid Yellow 1 does not induce gene mutations on bacterial and mammalian cells or possibly clastogenic effects on mammalian cells *in vitro*.
A possible clastogenic/aneugenic effect (p=0.05) at the highest dose was seen in males in an *in vivo* mouse bone marrow micronucleus study. The data provided are not sufficient to express a conclusive evaluation of the potential mutagenicity/genotoxicity of Acid Yellow 1.

The sensitivity of the skin painting carcinogenicity test is low and it is unlikely that it would have identified a carcinogenic potential.

2.13. References

1. Certificate of analysis (Ext. D&C Yellow 7); Cosmital SA, Marly, Switzerland; October 29, 2002
2. Ext. D&C Yellow 7 (C.I. 10316): 14-day oral toxicity (gavage) study in the rat; RCC Ltd, 4452 Itingen, Switzerland; RCC Project 740632; October 14, 1999
3. Acid Yellow 1: 13 week oral (gavage) toxicity study in the Wistar rat; RCC Ltd, CH-4452 Itingen, Switzerland; Study number 846105; August 12, 2003
4. Assessment of the eye irritation potential of raw material no. 23122 by cytotoxicity measurement in the neutral red uptake assay (NRU) on human keratinocytes (HaCaT); Cosmital SA, CH-1723 Marly, Switzerland; Study number NU 144; April 15, 2003
5. Ext. D&C Yellow 7, WR23122 (Acid Yellow 1, CI 10316) – Local lymph node assay; MDS Pharma Services, F-69210 Saint Germain sur l'Arbresle, France; Study number 762/028; Draft no.1. Datum?
6. Ext. D&C Yellow 7 (C.I. 10316): Contact hypersensitivity in albino Guinea pigs maximization-test; RCC Ltd, CH-4414 Füllinsdorf, Switzerland; RCC Project 740608; February 1, 2000
7. Acid Yellow 1 (CI 10316): Prenatal developmental toxicity study in the rat; RCC Ltd, CH-4452 Itingen, Switzerland; RCC study number 846299; June 24, 2003
8. Skin permeability in vitro absorption through porcine ear skin with Ext. D&C Yellow 7 (C.I. 10316); RCC-CCR, D-64380 Rossdorf, Germany; Project 641103; January 30, 2001
9. Salmonella typhimurium and Escherichia Coli reverse mutation assay with Ext. D&C Yellow 7 (C.I. 10316), RCC Cytotest Cell research GmbH, D-64380 Rossdorf, Germany, study number 641101, June 09, 2000
10. Cell mutation assay at the thymidine kinase locus ($TK^{+/-}$) in mouse lymphoma L5178Y cells with Ext. D&C Yellow 7 (C.I. 10316), RCC Cytotest Cell research GmbH, D-64380 Rossdorf, Germany, study number 641102, August 07, 2000
11. Micronucleus assay in bone marrow cells of the mouse with Acid Yellow 1 / 23122 / CI 10316, RCC Cytotest Cell research GmbH, D-64380 Rossdorf, Germany, study number 766000, March 16, 2003
12. Carson S. Skin painting studies in mice on 11 FD&C and D&C Colors: FD&C Green No. 3, Red No. 2, Red No. 4, Yellow No. 6, and External D&C No. 7, D&C Orange No. 4, Violet No. 2, Red No. 17, Red No. 34, and Yellow No. 7; J. Toxicol.-Cut & Ocular Toxicol. 3(3), 309-331 (1984)

13. LAN-Analysenbericht Studien-Nr A2002/334; Oct. 29, 2002. Wella AG; D-64274 Darmstadt
14. LAN-Analysenbericht Draft Studien-Nr. A2003/315-001+002; Sept. 24, 2003 Wella AG; D-64274 Darmstadt
15. LAN-Analysenbericht Studien-Nr A2002/313-003; Oct. 23. 2002. Wella AG; D-64274 Darmstadt
16. LAN-Analysenbericht Studien-Nr A2003/060; Feb. 21, 2003. Wella AG; D-64274 Darmstadt
17. LAN-Analysenbericht Studien-Nr A2003/297-001; Aug. 28, 2003. Wella AG; D-64274 Darmstadt
18. Certificate of analysis by LCW Wackherr; July 22, 1997
19. Stability in formulation; Sept. 22, 2003-09-25. Wella AG; D-64274 Darmstadt

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. There is no information for use in combination with hydrogen peroxide.

Before any further consideration, the following information is required :

* complete physico-chemical characterisation of the test substances used;

* irritation studies;

* percutaneous absorption study in accordance with the SCCNFP Notes of Guidance, if used in an oxidising environment;

* data on the genotoxicity/mutagenicity following the relevant SCCNFP-opinions and in accordance with the Notes of Guidance.

* final dossiers of on-going studies