



Scientific Committee on Consumer Products

SCCP

# OPINION ON

# Acid Yellow 1

COLIPA n° B1


The SCCP adopted this opinion at its 18th plenary of 16 December 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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http://ec.europa.eu/health/ph_risk/risk_en.htm

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## 1. BACKGROUND

Submission I for Acid Yellow 1, with the chemical name Disodium 5,7-dinitro-8-oxido-2-naphthalenesulfonate, was submitted in September 2003 by COLIPA[1].

The Scientific Committee on Consumer Products and Non Food Products intended for Consumers (SCCNFP) adopted on 23 April 2003 opinion SCCNFP/0783/04 with the conclusion, 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*".

This current submission II, submitted by COLIPA in July 2005, is an update of the submission I for Acid Yellow 1.

Acid Yellow 1 is identical with CI 10316 also used as a colouring agent allowed for use 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.

Acid Yellow 1 is used as a direct dye in oxidative hair dye formulations at a maximum concentration on the scalp at 1.0% and in non-oxidative hair dye formulations at a maximum concentration on the scalp at 0.2%

Submission II presents updated scientific data on the above mentioned substance in line with the second step of the strategy for the evaluation of hair dyes (http://europa.eu.int/comm/enterprise/cosmetics/doc/hairdyestrategyinternet.pdf) within the framework of the Cosmetics Directive 76/768/EEC.

## 2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Products (SCCP) consider Acid Yellow 1 safe for consumers when used in oxidative hair dye formulations with a concentration on the scalp of maximum 1.0% taking into account the scientific data provided?*

2. *Does the SCCP consider Acid Yellow 1 safe for consumers when used in non-oxidative hair dye formulation with a concentration on the scalp of maximum 0.2% taken into account the scientific data provided?*

3. *And/or does the SCCP recommend any restrictions with regard to the use of Acid Yellow 1 in any hair dye formulations?*

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

## 3. OPINION

3.1. Chemical and Physical Specifications

### 3.1.1. Chemical identity

#### 3.1.1.1. Primary name and/or INCI name

Acid Yellow 1 (INCI name)

#### 3.1.1.2. Chemical names

8-Hydroxy-5,7-dinitro-2-naphthalenesulfonic acid disodium salt
2-Naphthalenesulfonic acid, 8-hydroxy-5,7-dinitro-, disodium salt (CA INDEX NAME, 9CI)
Disodium 5,7-dinitro-8-oxido-2-naphthalenesulfonate (IUPAC)

#### 3.1.1.3. Trade names and abbreviations

D&C Yellow 7
Ext. D&C Yellow No. 7
Japan Yellow 403-1
Japan Yellow 403

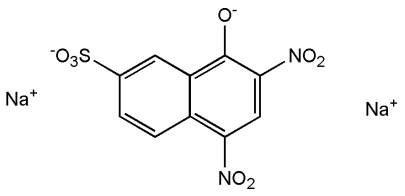
CI 10316
COLIPA n° B001

Citronin A
Flavianic acid sodium salt
Naphthol Yellow S

#### 3.1.1.4. CAS / EINECS number

CAS: 846-70-8
EINECS: 212-690-2

#### 3.1.1.5. Structural formula




#### 3.1.1.6. Empirical formula

Formula: $C_{10}H_6N_2O_8S.2Na$

### 3.1.2. Physical form

Yellow powder

### 3.1.3. Molecular weight

Molecular weight: 358.19 g/mol

### 3.1.4. Purity, composition and substance codes

| Description of sample | Batch 14067<br>FDA certified lot AG6738 | Batch B3943<br>FDA certified lot AL5546 |
|---|---|---|
| **References of Analyses** | **A 2002/334<br>A 2003/315-001<br>A 2005/293** | **A 2002/313-003<br>A 2003/060<br>A 2003/297-001<br>A 2003/315-002** |
| **NMR content / %, w/w** | 88.3 | 86.3 |
| **HPLC purity / area %*** | | |
| **210 nm** | 99.4 | - |
| **254 nm** | 99.4 | 99.6 |
| **430 nm** | 99.9 | 99.8 |
| **Sum of volatile matter and chlorides and sulphates** | 0.5% volatile matters**<br>Chloride 1.39%, w/w<br>Sulphate < 0.05%, w/w | 0.9% volatile matters**<br>Chloride 1.0%, w/w<br>Sulphate: 0.69%, w/w |
| **Loss on drying ( %, w/w)** | 5.57 | 5.8 |
| **Water content ( %, w/w)** | 7.1 | 6.9 |
| **Sulphated ash ( %, w/w)** | 38.7 | 38.9 |
| **Total colour content ( %, w/w)** | 89 ** | 89 ** |
| **Impurities:** | | |
| **1-Naphthol ( %, w/w)** | < 10 ppm* | < 10 ppm* |
| **2,4-Dinitro-1-naphthol ( %, w/w)** | < 20 ppm* | < 20 ppm* |
| **Lead** | < 10 ppm** | < 10 ppm** |
| **Arsenic** | < 3 ppm** | < 3 ppm** |
| **Mercury** | < 1 ppm** | < 1 ppm** |
| **Iron** | 9 ppm | 3 ppm |

* Below detection limit; indicated value shows detection limit
** From certificate of analysis
*** HPLC conditions: Column: Purospher C 18e, 100, 5µm, 250x4 mm; Eluent: 15% acetonitrile/ 85% 0.02M KH2PO4 sol. pH= 6.1; Flow: 1 ml/min

### 3.1.5. Impurities / accompanying contaminants

See point 3.1.4

### 3.1.6. Solubility

| | | | |
|---|---|---|---|
| Water: | 42.2 g/L (20°C, pH 7.75) | (EU Method A.6) | Ref. 4 |
| Acetone / water 1:1: | 4.1 weight % (pH 8.9) | | Ref. 1 |
| DMSO: | > 10 weight % | | Ref. 1 |

### 3.1.7. Partition coefficient (Log $P_{ow}$)

| | | | |
|---|---|---|---|
| Log Po/w: | - 0.24 (pH 6.96, 25°C) | (EU Method A.8) | Ref. 2 |

### 3.1.8. Additional physical and chemical specifications

| | | | |
|---|---|---|---|
| Particle size distribution: | mean particle diameter: 235 µm (CIPAC MT59) | | Ref. 3 |
| pH-value: | 7.75 (saturated aqueous solution, 20 °C) | | Ref. 4 |
| Melting point: | not detectable (decomposition starting at 310 °C) | (EU – A.1) | Ref. 6 |
| Boiling point: | not detectable (decomposition starting at 310 °C) | (EU – A.2) | Ref. 6 |
| Density: | 1.920 g/ml (20 °C) | (EU - A.3) | Ref. 7 |
| Vapour pressure: | < 1.0 exp – 7 hPa (20 °C) | (EU - A.4) | Ref. 8 |
| Surface tension (in water): | 71.1 mN/m (19.8 – 19.9 °C) | (EU - A.5) | Ref. 9 |
| Flammability (solids): | not highly flammable | (EU - A.10) | Ref. 10 |

| | | | |
|---|---|---|---|
| Explosive properties: | not explosive | (EU - A.14) | Ref. 11 |
| Rel. self-ignition temperature: | 341 °C | (EU - A.16) | Ref. 12 |
| UV_Vis spectrum (200-800 nm): | / | | |

### 3.1.9. Homogeneity and Stability

3 - 30 mg/ml formulations in 1% CMC were stable for up to test period of 7 days (variation in concentrations <10%)

Acid Yellow 1 was stable for 7 days in 6% (w/v) aqueous solution (recovery 983 - 98.6%). It was stable for 7 days in 3.3% (w/v) solution in acetone:water (1:1) (recovery 99 - 103%).
Acid Yellow 1 was stable for 7 days in 10% (w/v) solution in DMSO (recovery 97.6 - 99.9%)

**General Comments to physico-chemical characterisation**

- The stability of Disperse Red 17 in oxidative hair dye formulations has not been demonstrated.

## 3.2. Function and uses

*a) Oxidative Hair Colorants*
Acid Yellow 1 is used as a direct dye in oxidative hair dye formulations at a maximum on-head concentration of 1%.

*b) Semipermanent Hair Colorants*
Acid Yellow 1 is proposed to be used in semi-permanent hair dye formulas at a maximum on-head concentration of 0.2% in the finished cosmetic product.

## 3.3. Toxicological Evaluation

### 3.3.1. Acute toxicity

#### 3.3.1.1. Acute oral toxicity

Acute toxicity data are not available. The applicant considered that, since a repeated dose 14-day oral toxicity study in rats and a 90-day oral rat study are available, which shows an effect at 30 mg/kg bw/day, acute toxicity data would not support the evaluation of the toxicity potential of the substance for the intended use conditions.
Since oral administration of repeated doses over 14 days up to 1000 mg/kg bw did not induce any relevant systemic toxicity, the acute toxicity potential of Acid Yellow 1 is considered to be low. This consideration is further supported by the results of the micronucleus assay. In this assay, no lethality was observed for males up to 1800 mg/kg bw and for females up to 2000 mg/kg bw.

#### 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

**SkinEthic human reconstituted epidermis (RHE) model, age 17 days, 0.5 cm²**

Guideline: /
Species: /
Group: /
Substance: Acid Yellow 1 (CI 10316)
Batch: B3943
Purity: 99.6 area%, (HPLC, 254 nm),
Dose: neat (powder) and 2% (w/w in water)
Vehicle: water
GLP: not in compliance
Study period: 2005

The aim of this study was to assess *in vitro* the skin irritation potential of the test item using a reconstituted human epidermis model. The assay is based on the measurement of tissue viability after application of the test item onto the surface of *in vitro* reconstituted epidermis for 15 min, followed by a rinsing step and a post-treatment incubation of 42 h, by means of the 3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) reduction assay. This assay assesses cellular mitochondrial dehydrogenase activity.
A decrease in MTT reduction capacity and changes in tissue morphology were used as indicators of potential irritancy.

Results
In this MTT assay, Acid Yellow 1 resulted in 96% viability when applied neat, and 93% when applied at 2% in water. Interference of the colour of the test item with MTT did not occur. Histological evaluation of the treated tissues did not show any significant damaging effect in the treated cultures in comparison to the negative control cultures at both concentrations tested.

Conclusion
According to this prediction model, based on the percent viability measured in the MTT assay, Acid Yellow 1 is classified as non-irritant to human skin (% MTT viability > 50%), when tested neat and at 2% in water. The histological evaluation confirmed the absence of cytotoxicity.

Ref.: 17

Comment
Although the MTT assay is not yet validated, it can be anticipated that irritant effects in human skin should not occur at the intended maximum use concentration of 1% in hair dye formulations.
The results from a Guinea pig maximisation test (ref. 22) support the conclusion that Acid Yellow 1 is not irritant to the skin (no irritant effects seen up to 10%).

### 3.3.2.2. Mucous membrane irritation

**Normal human keratinocytes from the cell line HaCaT**

Guideline: /
Species: /
Group: /
Substance: Acid Yellow 1

| | |
|---|---|
| Batch: | B3943 |
| Purity: | 99.6 area% (HPLC, 254 nm), |
| Dose: | 0 to 10,000 µg/ml |
| Vehicle: | KGM® (serum-free keratinocyte growth medium), Clonetics CC-3101 (KBM®, keratinocyte basal medium) supplemented with CC-4131 (KGM® SingleQuots®, pre-measured, single-use aliquots of growth factors and antimicrobials |
| Diluting agent: | KGM® (the solubility of Acid Yellow 1 in KGM® was not stated) |
| GLP: | not in compliance |
| Study period: | 10 – 28 March 2003 |

The Neutral Red Uptake (NRU) assay assesses the eye irritation potential of the test item by measuring its cytotoxicity. 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, and the concentration causing 50 % reduction in neutral red uptake in treated cells compared to untreated control cells (NRU-50) was determined.

Results
In a dose-range-finding assay, the NRU-50 value of the test item was identified between 10,000 and 3162 µg/ml. Based on this result, eight doses ranging from 10,000 to 681.3 µg/ml were selected for the two definitive NRU assays.
The median NRU-50 value obtained in two independent assays with the test item was 6916 µg/ml.

Conclusion
Under the test conditions used, it is concluded that Acid Yellow 1 is non-irritant (NRU-50 ≥ 750 µg/ml).

Ref.: 18

Comment
This study was performed using an in-house method instead of the COLIPA prediction model:
- human keratinocytes from the cell line HaCaT were used instead of the Balb/c 3T3 mouse fibroblasts;
- the treatment was performed in serum-free culture medium.

**SkinEthic human corneal epithelial (HCE) model**

| | |
|---|---|
| Guideline: | / |
| Species: | / |
| Group: | / |
| Substance: | Acid Yellow 1 |
| Batch: | B3943 |
| Purity: | 99.6 area % (HPLC, 254 nm), |
| Dose: | 1, 2 and 5% (w/w in water) |
| Vehicle: | water |
| GLP: | not in compliance |
| Study period: | 5 October – 10 November 2005 |

The aim of this study was to assess *in vitro* the acute eye irritation potential of the test item using the reconstituted human corneal epithelium (HCE) model (age 5 days, 0.5 $cm^2$), which is based on the measurement of cytotoxicity in reconstituted human epithelial cultures after topical exposure to the test item for different treatment times (time-course protocol), by means of the colorimetric MTT reduction assay. Histological evaluation of the tissues at the end of the treatment was also performed.

A model, based on the viability of treated tissues expressed in percent of a negative control, was used to determine the eye irritation potential. In the MTT assay, the viability was measured at the end of each treatment period and the Effective Time-50 (ET-50), which is the time of exposure required to reduce the tissue viability of treated cultures to 50 % of untreated controls (negative control), was determined.

Results
Acid Yellow 1 did not induce any significant decrease in cell viability in the MTT assay, resulting in viabilities > 90 % at all time points and ET-50 values reported as > 180 min at the three concentrations tested. Interference of the colour of the test item with MTT did not occur. Histological examination showed that the test item did not cause damage to the tissue structures, when tested at 1, 2 and 5 % in water, even after the longest exposure time of 180 min.

Conclusion
In this model, based on the percent viability in MTT, Acid Yellow 1 was non-irritant (viability after 10 min treatment > 50 %) when tested at 1, 2 and 5 % in water.

Ref.: 20

Comment
The above methods have not been formally validated for the assessment of cosmetic ingredients.
The SkinEthic-study was performed according to the protocol evaluated in a recent multi-centre prevalidation study (Reference 19) for the assessment of the ocular irritation potential of chemicals using the human corneal epithelium model, which was designed to prepare the test model for further progression to a formal ECVAM validation study.

In conclusion, no eye irritation potential of Acid Yellow 1 was observed, either in the NRU assay with HaCaT cells or in the human corneal epithelium model (HCE) model. Eye irritating effects are unlikely to occur at the intended maximum use concentration of 1 % in hair dye formulations.

### 3.3.3. Skin sensitisation

**Local Lymph Node Assay (LLNA)**

| | |
|---|---|
| Guideline: | OECD 429 |
| Species: | Mouse: CBA/J |
| Group: | 5 females per dose and vehicle |
| Substance: | Acid Yellow 1 |
| Batch: | B3943 |
| Purity: | 99.6 area % (HPLC, 254 nm) |
| Dose: | 0.3, 1, 3, 10% in DMSO<br>0.3, 1, 3, 4.1% in acetone/water (1:1) mixed with olive oil (4:1) |
| Vehicle: | DMSO, acetone/water in olive oil |
| Control: | p-phenylenediamine (1% in DMSO) |
| GLP: | in compliance |
| Study period: | 15 - 20 January 2003 |

Acid Yellow 1 was tested in different concentrations (0.3, 1, 3, 10% (w/v)) in DMSO as well as (0.3, 1, 3 and 4.1% (w/v)) in acetone/water (1:1) mixed with olive oil (4:1). 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 ear.
All animals were killed on day 5 for assessment of cell proliferation via $^{3}$H-thymidine incorporation.

Results

In DMSO: 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): 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 PPD (p-phenylenediamine) was positive in the local lymph node assay, as there was at least a 3-fold increase in isotope incorporation in the draining auricular lymph nodes relative to the vehicle (DMSO). The mean stimulation index was 6.5 at the concentration of 1%.

Conclusion
The test item did not show skin sensitising potential under the experimental conditions using the two tested vehicles.

Ref.: 21

**Guinea Pig Maximization test**

| | |
|---|---|
| Guideline: | OECD 406 |
| Species: | Ibm: GOHI; SPF-quality guinea pigs |
| Group: | 15 females (10 test group and 5 control group) |
| Substance: | D&C Yellow 7 |
| Batch: | 14067, lot AG6738 |
| Purity: | 99.4 area % (HPLC, 254 nm) |
| Dose Induction: | Intradermal 5% in 1% CMC (carboxymethylcellulose) in an emulsion of Freund's Complete Adjuvant (FCA) / physiological saline. Epidermal 50% in 1% CMC |
| Dose Challenge: | 10% in 1% CMC |
| Control: | negative control; non-contemporaneous positive control using mercaptobenzothiazole |
| GLP: | in compliance |
| Study period: | 15 September – 11 October 1999 |

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 Freund's Complete Adjuvant (FCA) / physiological saline. The epidermal induction of sensitisation was conducted for 2 days 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. The concentration of 10% was chosen as the maximum non-irritating concentration of the test item.

Cutaneous reactions were evaluated at 1 and 2 days after removal of the dressing.

Results
All test animals showed discrete/patchy to moderate/confluent erythema at the 1 and 2 day reading after the challenge treatment with D&C Yellow 7 at 10% (w/w) in 1% CMC. No skin effect was observed in the control group.

Conclusion
Based on the results, the test item is a skin sensitizer under the described test conditions.

Ref.: 22

Comment
Acid Yellow 1 is an extreme skin sensitiser according to the Magnusson & Kligman classification. The negative outcome of the LLNA may possibly be explained by the relatively low maximum concentrations used.

### 3.3.4. Dermal / percutaneous absorption

**Pig skin *in vitro*, non-oxidative formulation**

| | |
|---|---|
| Guideline: | OECD 428 |
| Tissue: | Porcine back and flank skin (thickness: mean 1000 µm). 3 donors: 1 female, 2 Male |
| Group size: | five for the formulation containing the test item and one for the blank formulation |
| Diffusion cells: | Diffusion Teflon-chambers |
| Skin integrity: | tritiated water. |
| Test substance: | Acid Yellow 1 |
| Batch: | B3943 |
| Purity: | 99.6 area% (HPLC at 254 nm) |
| Test item: | 0.2% in a typical non-oxidative hair dye formulation |
| Doses: | 100 mg/cm² of hair dye formulation |
| Receptor fluid: | physiological phosphate buffer containing NaCl and antibiotics |
| Solubility receptor fluid: | 62.48 mg/ml |
| Stability: | / |
| Method of Analysis: | HPLC. The limit of detection was found to be 5 ng per injection and the limit of quantification was 20 ng per injection |
| GLP: | In compliance |
| Study period: | 26 September – 5 October 2005 |

The skin absorption of Acid Yellow 1 in a non-oxidative hair dye formulation was investigated with pig skin prepared from the back and the flanks. 400 mg of the formulation (= 100 mg/cm²) containing 0.2% Acid Yellow 1 was applied once to the skin samples (4 cm²). Diffusion Teflon-chambers were used. The receptor solution was pumped through the receptor chamber at a rate of 5 ml/h. Six chambers were investigated.

Sixty minutes after substance application, the test item was removed by washing the skin twice with 4 ml water, then once with 4 ml of a shampoo-formulation (diluted to approximately 14 %), and again twice with water. These rinsing solutions were combined and the amount of dye was determined by HPLC.
Fractions of the receptor fluid were collected after 16, 24, 40, 48, 64 and 72 hours, concentrated by solid phase extraction immediately and analysed by HPLC. At termination of the experiment, the skin was heat-treated and the "upper skin" (stratum corneum and upper stratum germinativum) mechanically separated from the "lower skin" (lower stratum germinativum and upper dermis). Both skin compartments were extracted separately and the dye content was quantified by means of HPLC.

Results
The total balance (total recovery) was 106.57 ± 3.06% of the applied dose.
The majority of the applied dose was found in the rinsing solutions (106.53 ± 3.06% of the applied dose) after the 60 min application period. After the experimental observation period of 72 h, the amounts in the majority of the receptor fluid samples were below the respective limit of detection of 0.0025 µg/cm². Only in four fractions of the first sampling period (16 h), the values were at the limit of quantification of 0.01 µg/cm² or slightly above, i.e. 0.012 and 0.014 µg/cm², respectively. Thus, the total amount penetrated into the receptor fluid was 0.023 ± 0.005 µg/cm². The amounts of Acid Yellow 1 found in the upper skin and in the

lower skin after 72 h were below the respective limit of detection of 0.025 µg/cm² for the skin compartments.
Since the amounts measured in all analysed fraction were close to or below the limit of detection, the following conservative assumption was used: The amounts found in the receptor fluid and in both skin layers including the stratum corneum were used to calculate the penetration rate i.e. 0.073 ± 0.005 µg/cm² [0.023 µg/cm² for receptor fluid + 0.025 µg/cm² for skin upper + 0.025 µg/cm² for lower skin].

Conclusion
Under the described test conditions a maximum amount of 0.073 ± 0.005 µg/cm² of Acid Yellow 1 (n=5, three donors; receptor fluid + lower skin + upper skin) was considered as bioavailable. As a conservative assumption, the concentration found in the stratum corneum is included.

Ref.: 24

Comment
The study was not performed according to the SCCP Notes of Guidance. Only 5 chambers were used and 100 mg/cm² of formulation were applied in the test. Any absorption was close to the limit of detection. An $A_{max}$ of 0.078 µg/cm² (receptor fluid + lower skin + upper skin) was obtained under non-oxidative conditions.

**Pig skin *in vitro*, oxidative formulation**

| | |
|---|---|
| Guideline: | OECD 428 |
| Tissue: | Porcine back and flank skin (thickness: mean 1000 µm). 3 donors: 1 female, 2 Male |
| Group size: | four for the formulation containing the test item and one for the blank formulation |
| Diffusion cells: | Diffusion Teflon-chambers |
| Skin integrity: | tritiated water. |
| Test substance: | Acid Yellow 1 |
| Batch: | B3943 |
| Purity: | 99.6 area% (HPLC at 254 nm) |
| Test item: | 1% in a typical oxidative hair dye formulation |
| Doses: | 100 mg/cm² of hair dye formulation |
| Receptor fluid: | physiological phosphate buffer containing NaCl and antibiotics |
| Solubility receptor fluid: | 62.48 mg/ml |
| Stability: | / |
| Method of Analysis: | HPLC. The limit of detection was found to be 5 ng per injection and the limit of quantification was 20 ng per injection |
| GLP: | In compliance |
| Study period: | 19 - 26 September 2005 |

The skin absorption of Acid Yellow 1 in a oxidative hair dye formulation was investigated with pig skin prepared from the back and the flanks. 400 mg of the formulation (= 100 mg/cm$^2$) containing 1% Acid Yellow 1 was applied once to the skin samples (4 cm²). Diffusion Teflon-chambers were used. The receptor solution was pumped through the receptor chamber at a rate of 5 ml/h. Six chambers were investigated.

Sixty minutes after substance application, the test item was removed by washing the skin twice with 4 ml water, then once with 4 ml of a shampoo-formulation (diluted to approximately 14 %), and again twice with water. These rinsing solutions were combined and the amount of dye was determined by HPLC.
Fractions of the receptor fluid were collected after 16, 24, 40, 48, 64 and 72 hours, concentrated by solid phase extraction immediately and analysed by HPLC. At termination of the experiment, the skin was heat-treated and the "upper skin" (stratum corneum and

upper stratum germinativum) mechanically separated from the "lower skin" (lower stratum germinativum and upper dermis). Both skin compartments were extracted separately and the dye content was quantified by means of HPLC.

Results
The total balance (total recovery) was 102.32 ± 2.74 % of the applied dose.
The majority of the applied dose of Acid Yellow 1 remained on the skin surface (102.32 ± 2.74% of the applied dose or 1.023 ± 0.027 mg/cm²) and was recovered in the rinsing solutions.
After 72 h, 0.036 ± 0.01 µg/cm² were found in the receptor fluid samples. The amounts of Acid Yellow 1 found in the upper skin and in the lower skin after 72 h were below the respective limit of detection of 0.025 µg/cm$^2$ for the skin compartments. Since the evaluation of the penetration kinetics did not allow to exclude a potential contribution of Acid Yellow 1 from a reservoir in the skin layers (depot effect), the following conservative assumption was used: The amounts found in the receptor fluid and in both skin layers including the stratum corneum were used to calculate the penetration rate i.e. 0.086 ± 0.010 µg/cm² [0.036 µg/cm² for receptor fluid + 0.025 µg/cm² for skin upper + 0.025 µg/cm$^2$ for lower skin].

Conclusion
Under the described test conditions a maximum amount of 0.086 ± 0.010 µg/cm$^2$ of Acid Yellow 1 (n=4, three donors; receptor fluid + lower skin + upper skin) was considered as bioavailable. As a conservative assumption, the concentration found in the stratum corneum is included.

Ref.: 25

Comment
The study was not performed according to the SCCP Notes of Guidance. Only 4 chambers were used and 100 mg/cm² of formulation were applied in the test. Any absorption was close to the limit of detection. An Amax of 0.096 µg/cm² (receptor fluid + lower skin + upper skin) was obtained under oxidative conditions.

## 3.3.5. Repeated dose toxicity

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

***Taken from SCCNFP/0783/04***

| | |
|---|---|
| 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: | 14067 |
| Dose levels: | 0, 100, 300 and 1000 mg/kg bw/day, by gavage |
| Exposure period: | 14 days |
| GLP: | not in compliance |
| Study period: | 10 – 24 August 1999 |

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.: 15

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

***Taken from SCCNFP/0783/04***

| | |
|---|---|
| Guideline: | OECD 408 (1998) |
| Species/strain: | Rat, HanBrl: WIST (SPF) |
| Size: | 10 males and 10 females per dose |
| Test item: | Acid Yellow 1 |
| Batch: | 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: | not in compliance |
| Study period: | 21 November 2002 – 28 February 2003 |

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.

Conclusion
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.: 16

| 3.3.5.3. Chronic (> 12 months) toxicity |
|---|

No data submitted

## 3.3.6. Mutagenicity / Genotoxicity

| 3.3.6.1 Mutagenicity / Genotoxicity *in vitro* |
|---|

**Bacterial Reverse Mutation Test**

***Taken from SCCNFP/0783/04***

| | |
|---|---|
| Guideline: | OECD/471 (1997) |
| Species/Strain: | *S. typhimurium (*TA1535, TA1537, TA98, TA100); *E. coli* (WP2 uvrA) |
| Test item: | Ext D&C Yellow 7 (CI 10316) |
| Batch: | 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 |
| Study period: | 24 August – 6 September 1999 |

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.: 27

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

***Taken from SCCNFP/0783/04***

| | |
|---|---|
| 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: | 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-mix: 250, 500, 100, 2000, 4000 µg/ml (2 experiments)<br>with S9-mix: 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);<br>(without metabolic activation) methyl methane sulphonate (MMS) |
| GLP: | in compliance |
| Study period: | 21 September – 8 November 1999 |

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-mix) and for 4h (+S9-mix). 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-mix) one culture. The mutant frequency (MF) of MMS (-S9-mix) positive control was $197x10^6$ cells (control: 87) for small colonies and $105 \times 10^6$ cells (control: 24) for large colonies. The MF of 3-MC (+S9-mix) 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-mix 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.: 28

***In vitro* Micronucleus Test**

| | |
|---|---|
| Guideline: | Draft OECD guideline no. 487 (2004) |
| Species/strain: | Human peripheral blood lymphocytes from two to three male donors in each trial |
| Replicates: | Two cultures per concentration and positive controls (4 for the negative control), 3 concentrations analysed<br>Two independent experiments using pooled blood |
| Test Substance: | Acid Yellow 1 |
| Batch: | B3943 |
| Purity: | 99.4 area% (HPLC, 254 nm) |
| Concentrations: | Exp. I: with S9-mix: 2500, 3000 and 3582 µg/ml<br>without S9-mix: 1500, 2500 and 3582 µg/ml<br>Exp. II: with S9-mix and without S9-mix: 2500, 3000 and 3582 µg/ml |
| Treatment: | Experiment I: 24 hours mitogen stimulation (PHA)<br>With S9-mix: 3 hours treatment 28 hours recovery<br>Without S9-mix: 20 hours treatment 45 hours recovery<br>Harvest time 72 hours<br>Experiment II: 48 hours mitogen stimulation (PHA)<br>With S9-mix: 3 hours treatment 28 hours recovery<br>Without S9-mix: 20 treatment 45 hours recovery<br>Harvest time 96 hours |
| GLP: | In compliance |
| Study period: | 4 October – 8 November 2005 |

Acid Yellow 1 was examined for its clastogenic and aneugenic potential in the in vitro micronucleus assay using duplicate human lymphocyte cultures prepared from pooled blood of two healthy male donors. Two independent assays were performed with and without metabolic activation (S9-mix from the liver of Aroclor induced male Sprague Dawley rats).
In the cytotoxicity range-finding experiment, the test article was dissolved in dimethyl sulphoxide and the highest dose tested was 1000 µg/mL. However, no evidence of cytotoxicity or post-treatment precipitation was observed in cultures treated with the test article. Consequently, the test article was dissolved in purified water and the highest dose level used, 3582 µg/mL, was equivalent to 10 mM.

Results
The highest test concentration to be evaluated in experiment I and II was 3582 µg/ml (equivalent to 10 mM) without inducing cytotoxicity.
In experiment 1 (with 24 hour PHA stimulation prior to treatment) no statistically significant increase in the frequencies of micronucleated binucleate (MNBN) cells were observed either in the absence or in the presence of S9-mix. The MNBN cell frequency of all Acid Yellow 1 treated cultures fell within historical negative control ranges.
In experiment 2 (48 hour PHA stimulation prior to treatment): Treatment of cells in the absence of S9-mix resulted in frequencies of MNBN cells which were significantly higher than those observed in concurrent vehicle control cultures at the intermediate concentration analysed (3000 µg/mL). However, the MNBN cell frequency of all Acid Yellow 1 treated

cultures fell within normal ranges, therefore this observation was not considered biologically relevant. In the presence of S9-mix there was also an increase in frequencies of MNBN cells which were significantly higher than those observed in concurrent vehicle control cultures at the intermediate concentration analysed (3000 μg/mL). The MNBN cell frequencies of both replicate cultures treated at 3000 μg/mL marginally exceeded the 95% reference range of the normal historical range for male donors. However, one of these MNBN cell frequency values was within the observed normal range and the other was only marginally in excess of the observed range. Furthermore, the increase was not concentration related (no increase in mutation frequency at the highest tested concentration) and no evidence of cytotoxicity was observed at any concentration analysed up to the maximum recommended concentration (10mM). Therefore these findings were considered of no biological relevance. The negative and positive control data fell within the normal range and demonstrated the sensitivity and the validity of the test.

Conclusion
It is concluded that Acid Yellow 1 did not induce micronuclei in cultured human peripheral blood lymphocytes either in the absence or in the presence of S9-mix at concentrations up to 10 mM. Based on these results, Acid Yellow 1 is evaluated as non clastogenic and/or aneugenic in this *in vitro* assay.

Ref.: 29

### 3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

**Mammalian Erythrocyte Micronucleus Test**

***Taken from SCCNFP/0783/04***

| | |
|---|---|
| Guideline: | OECD/474 (1997) |
| Species/Strain: | NMRI Mice |
| Group size: | 5 males /5 females / group dosed |
| Doses: | 12.5-25-50 mg/Kg bw |
| Test item: | Acid Yellow 1/23122/CI 10310 |
| Batch: | B 3943 (Goldmann) |
| Purity: | 99.6% area (HPLC 254 nm)<br>99.8% area (HPLC 430 nm) |
| Stability: | not indicated by the Sponsor. Expiration date: October 2004 |
| Doses: | females 2x500; 2x1000; 2x2000 mg/kg bw<br>males 2x450; 2x900; 2x1800 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 |
| Study period: | 2 December 2002 – 16 January 2003 |

Results
There was no decrease in PCEs compared to control values at any of the tested doses in either sex, indicating that the test substance was not cytotoxic to the bone marrow. However, the urine of the animals was orange after treatment indicating its bioavailability. Furthermore, the treated animals showed clear signs of toxicity. There was no statistical significant increase ($P<0.05$) at any at the tested doses. There was a minor increase in the mid dose of males (0.13% micronucleated erythrocytes compared to 0.05 in the control animals). However, this increase was not dose related, well within the historical control range of the performing laboratory (0.01 - 0.21% for males) and not statistical significant ($P= 0.0514$) and therefore not considered biological relevant.

Conclusion

Under the test conditions used, Acid Yellow 1 was not clastogenic and/or aneugenic *in vivo*.
Ref.: 30

### 3.3.7. Carcinogenicity

***Taken from SCCNFP/0783/04***

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 cm² area. Survival, body weight, and palpable growth were followed for an 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.: 31

Comment
The bioassay had several limitations with regard to reporting and experimental design, and was not in line with relevant guidelines. The test substance was assessed in a mixture at a concentration of 1%. This study is considered to be of limited value in evaluating the carcinogenic potential of Acid Yellow 1 in humans.

### 3.3.8. Reproductive toxicity

#### 3.3.8.1. Two generation reproduction toxicity

No data submitted

#### 3.3.8.2. Teratogenicity

***Taken from SCCNFP/0783/04***

| | |
|---|---|
| Guideline: | OECD 414 (2001) |
| Species/strain: | Rat WIST HanBRL: WIST (SPF) |
| Size: | 96 mated females 24 per group" |
| Test item: | Acid Yellow 1, |
| Batch: | B3943 Goldmann |
| Purity: | 97.5% |
| Stability: | max 4h at 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 |
| Study period: | 18 November – 17 December 2002 |

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, dosed 450 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 or 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.: 23

### 3.3.9. Toxicokinetics

No data submitted

### 3.3.10. Photo-induced toxicity

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 Yellow 1**)
(Oxidative / permanent)

| | | | |
|---|---|---|---|
| **Maximum absorption through the skin** | **A (µg/cm$^2$)** | **=** | **0.096 µg/cm²** |
| **Skin Area surface** | **SAS (cm$^2$)** | **=** | **700 cm$^2$** |
| **Dermal absorption per treatment** | **SAS x A x 0.001** | **=** | **0.067 mg** |
| **Typical body weight of human** | | **=** | **60 kg** |
| **Systemic exposure dose (SED)** | **SAS x A x 0.001/60** | **=** | **0.0011 mg/kg bw** |
| **No observed adverse effect level (90-day, oral, rat)** | **NOAEL** | **=** | **30 mg/kg bw** |
| **Margin of Safety** | **NOAEL / SED** | **=** | **27273** |

**(Acid Yellow 1**)
(Non-oxidative / semi-permanent)

| | | | |
|---|---|---|---|
| **Maximum absorption through the skin** | **A (µg/cm$^2$)** | **=** | **0.078 µg/cm²** |
| **Skin Area surface** | **SAS (cm$^2$)** | **=** | **700 cm$^2$** |
| **Dermal absorption per treatment** | **SAS x A x 0.001** | **=** | **0.055 mg** |
| **Typical body weight of human** | | **=** | **60 kg** |
| **Systemic exposure dose (SED)** | **SAS x A x 0.001/60** | **=** | **0.0009 mg/kg bw** |
| **No observed adverse effect level (90-day, oral, rat)** | **NOAEL** | **=** | **30 mg/kg bw** |
| **Margin of Safety** | **NOAEL / SED** | **=** | **33333** |

Comment
Using the values of the *in vitro* dermal absorption study which had several shortcomings, a very high Margin of Safety is calculated. In this case, the dermal absorption study can be exceptionally accepted.

## 3.3.14. Discussion

*Physico-chemical properties*
Acid Yellow 1 is used in oxidative hair dye formulations at a maximum on-head concentration of 1 %. It is proposed to be used in semi-permanent hair dye formulas at a maximum on-head concentration of 0.2 % in the finished cosmetic product. The stability of Acid Yellow 1 in marketed products is not reported.

*General toxicity*
Based on the results of the 90-day study, 30 mg/kg bw/day of Acid Yellow 1 was established as the no observed adverse effect level (NOAEL).
The No Observable Adverse Effect Level (NOAEL) for maternal toxicity was set at 50 mg/kg bw/day and at 150 mg/kg bw/day for foeto-toxicity. There was no indication of teratogenic potential.

*Irritation / sensitisation*
Acid Yellow 1 is considered to be non-irritant to human skin (% MTT viability > 50%), when tested neat and at 2% in water, based on the percent viability measured in the MTT assay.

No eye irritation potential of Acid Yellow 1 was observed, either in the NRU assay with HaCaT cells or in the human corneal epithelium model (HCE) model. Eye irritating effects are unlikely to occur at the intended maximum use concentration of 1% in hair dye formulations.
Acid Yellow 1 is an extreme skin sensitiser according to the Magnusson & Kligman classification. The negative outcome of the LLNA may possibly be explained by the relatively low maximum concentrations used.

*Dermal absorption*
The *in vitro* dermal absorption study was not performed according to the SCCP Notes of Guidance. Too few chambers (4 and 5) were used and 100 mg/cm² of formulation were applied in the test. Any absorption was close to the limit of detection. An $A_{max}$ of 0.096 µg/cm² under oxidative conditions and of 0.078 µg/cm² under non-oxidative conditions was obtained. Using these values for calculation, a very high Margin of Safety is obtained. In this case, the dermal absorption study can be exceptionally accepted despite its shortcomings.

*Mutagenicity / genotoxicity*
Overall, Acid Yellow 1 was tested for gene mutation in bacteria and mammalian cells, structural and numerical chromosomal aberrations. Acid yellow 1 did not induce gene mutations in bacteria, it did not induce mutants in the mouse lymphoma assay and it was not clastogenic or aneugenic in the micronucleus assay *in vitro*. No clastogenic or aneugenic effect was observed in an *in vivo* micronucleus assay in bone marrow cells of mice. It is concluded that Acid Yellow 1 is not genotoxic either *in vivo* or *in vitro*.

*Carcinogenicity*
The bioassay had several limitations with regard to reporting and experimental design, and was not in line with relevant guidelines. The test substance was assessed in a mixture at a concentration of 1%. This study is considered to be of limited value in evaluating the carcinogenic potential of Acid Yellow 1 in humans.

## 4. CONCLUSION

The SCCP is of the opinion that, apart from the risks associated with the use of a sensitiser, the use of Acid Yellow 1 as an ingredient in oxidative hair dye formulations at a maximum on-head concentration of 1.0% and in non-oxidative hair dye formulations at a maximum on-head concentration of 0.2% does not pose a risk to the health of the consumer.

Acid Yellow 1 is considered to be a potent skin sensitiser.

The stability of Acid Yellow 1 in oxidative hair dye formulations has not been demonstrated.

## 5. MINORITY OPINION

Not applicable

## 6. REFERENCES

1. Umbricht, G.; Certificate of Analysis WR 23122; D&C Yellow 7; *COSMITAL SA*; 2002

2. Lange, J.; Partition Coefficient (n-octanol / water) using High Performance Liquid Chromatography (HPLC); *NOACK*; 2003
3. Lange, J.; Sieve Analysis; *NOACK*; 2004
4. Lange, J.; Water Solubility (Flask Method); *NOACK*; 2004
5. Lange, J.; Prediction of the pKa-value; *NOACK*; 2004
6. Smeykal, H.; Melting Point / Boiling Point; *SIEMENS AXIVA GMBH & CO. KG*; 2004
7. Lange, J.; Determination of the Density; *NOACK*; 2004
8. Franke, J.; Vapour Pressure; *SIEMENS AXIVA GMBH & CO. KG*; 2004
9. Lange, J.; Surface Tension; *NOACK*; 2004
10. Lange, J.; Flammability of Solids; *NOACK*; 2004
11. Smeykal, H.; Explosive Properties; *SIEMENS AXIVA GMBH & CO. KG*; 2004
12. Smeykal, H.; Auto-Flammability (Solids - Determination of relative self-ignition temperature); *SIEMENS AXIVA GMBH & CO. KG*; 2004
13. Smeykal, H.; Oxidizing Properties; *SIEMENS AXIVA GMBH & CO. KG*; 2004
14. Damkröger, G.; LAN-Analysenbericht; *COSMITAL SA*; 2002
15. Rosner, E.; Ext. D&C Yellow 7 (C.I. 10316); 14-day oral toxicity (gavage) study in the rat; *RCC*; 1999
16. Damme, B.; Knuppe, C.; Gretener, P.; Weber, K.; 13-Week Oral (Gavage) Toxicity Study in the Wistar Rat; *RCC*; 2003
17. Aliferis, M.; Skin irritation assay using the SkinEthic in vitro reconstituted human epidermis tissue model; *SKIN ETHIC LABORATORIES*; 2005
18. Faller, C.; 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*; 2003
19. Van Goethem, F.; Adriaens, E.; Alépée, N.; Straube, F.; De Wever, B.; Cappadoro, M.; Catoire, S.; Hansen, E.; Wolf, A.; Vanparys, P.; Prevalidation of a new in vitro reconstituted human cornea model to assess the eye irritating potential of chemicals; *TOXICOL. IN VITRO;* In press, 2005
20. Faller, C.; Assessment of the Eye Irritation Potential of Acid Yellow 1 (WR 18046/23122) by Cytotoxicity Measurement using reconstituted human corneal epithelium; *COSMITAL SA*; 2005
21. Christ, M.; Ext. D & C Yellow 7; WR23122 (Acid Yellow 1, CI 10316) - Local Lymph Node Assay; *MDS PHARMA SERVICES*; 2003
22. Arcelin, G.; Ext. D&C Yellow 7 (C.I. 10316) Contact hypersensitivity in albino guinea pigs Maximization -Test; *RCC*; 2000
23. Khalil, S.; Knuppe, C.; Prenatal Developmental Toxicity Study in the Rat; *RCC*; 2003
24. Sieber, T. P.; Cutaneous Absorption of 0.2% Acid Yellow 1 (=WR18046) in a typical hair dye formulation through pig skin in vitro; *COSMITAL SA*; 2005
25. Sieber, T. P.; Cutaneous Absorption of 1% Acid Yellow 1 (=WR18046) in a typical hair dye formulation with hydrogen peroxide through pig skin in vitro; *COSMITAL SA*; 2005
26. Simon, G. A.; Maibach, H. I.; The pig as an experimental animal model of percutaneous permeation in man: Qualitative and quantitative observations - An overview; *SKIN PHARMACOL. PHYSIOL.;* 13, *229-234;* 2000
27. Wollny, H.-E.; Salmonella Typhimurium and Escherichia Coli reverse mutation assay with Ext. D&C Yellow 7 (C.I. 10316); *RCC*; 2000
28. Wollny, H.-E.; Cell mutation assay at the thymidine kinase locus (TK +/-) in mouse lymphoma L5178Y cells with Ext. D&C Yellow 7 (C.I. 10316); *RCC*; 2000
29. Lloyd, M.; Acid Yellow 1 (WR 18046): Induction of micronuclei in cultured human peripheral blood lymphocytes; *COVANCE*; 2005
30. Honarvar, N.; Micronucleus assay in bone marrow cells of the mouse with Acid Yellow 1 / 23122 / CI 10316; *RCC-CCR; 1-50*; 2003
31. 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; *J. TOXICOL. CUTANEOUS OCUL. TOXICOL.;* 3, *309-331;* 1984