EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

HC Yellow n° 7

COLIPA n° B80

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

The SCCS adopted this opinion at its 8th plenary meeting

of 21 September 2010

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 Safety (SCCS), 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 (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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1. BACKGROUND

Submission I for HC Yellow n° 7 with the chemical name 1-(4'-Aminophenylazo)-2-methyl-4-(bis-2-hydroxyethyl)aminobenzene has been submitted in April 1996 by COLIPA [1, 2].

Submission II for HC Yellow n° 7 has been submitted in June 2001 by COLIPA [2].

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted at its 24th plenary meeting on 24-25 June 2003 the opinion (SCCNFP/0675/03, final) with the conclusion that:

"*The SCCNFP is of the opinion that the information submitted is inadequate to assess the safe use of the substance. Before any further consideration, the following information is required: proper analytical and physico-chemical data, e.g. characterisation of the purity/impurities of all the batches used, related health hazards of impurities, experimental data on stability, and sensitivity to light and moisture. Data on genotoxicity/mutagenicity following the relevant SCCNFP opinions and in accordance with the Notes of Guidance*."

Submission III for HC Yellow n° 7 was submitted by COLIPA in July 2005. According to this submission the substance is used in semi-permanent hair dye formulations at a maximum concentration of 0.25%.

Submission III 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 Safety (SCCS) consider HC Yellow n° 7 safe for use as a non-oxidative hair dye with an on-head concentration of maximum 0.25% taken into account the scientific data provided?*

2. *Does the SCCS recommend any further restrictions with regard to the use of HC Yellow n° 7 in any non-oxidative hair dye formulations?*

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association
[2] According to records of COLIPA

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

HC Yellow n° 7 (INCI name)

3.1.1.2. Chemical names

Ethanol, 2,2'[[4-[(4-aminophenyl)azo]-3-methylphenyl]imino]bis-
1-(4'-Aminophenylazo)-2-methyl-4-(bis-2-hydroxyethyl) aminobenzene

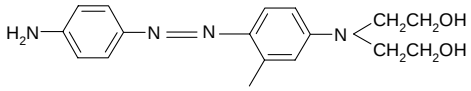
3.1.1.3. Trade names and abbreviations

Imexine® AA
COLIPA B080

3.1.1.4. CAS / EC number

CAS: 104226-21-3
EC: 146-420-6

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{17}H_{22}N_4O_2$

3.1.2. Physical form

Orange crystalline powder

3.1.3. Molecular weight

Molecular weight: 314.39 g/mol

3.1.4. Purity, composition and substance codes

Batch 0509831 and Op.56 were characterised by

- Infra-red and Vis. spectrophotometry,
- Mass and N.M.R. spectrometry,
- Elemental analysis

^{1}H and ^{13}C NMR spectra of batch 0509831 and Op.56 are in accordance with the proposed structure.
Mass spectra of batches 0509831 and Op.56 are compatible with the proposed structure.
IR spectra of batches 0509831, Op.56 and 0503175 are in accordance with the proposed structure.

Visible spectra of batches 0509831, Op.56, Op.26, Op.35, Op.T68 and Op.T77 are comparable.

Characterisation and composition of the batches of HC Yellow n° 7 used in various studies submitted

Batch No.	**0509831**	**Op. 56**	**Op. 35**	**Op. T68**	**Op. T77**	**Op. 26**	**0503175**
^{1}H and ^{13}C NMR Spectra, Mass spectrum, IR spectrum	In accordance with the proposed structure						In accordance with the proposed structure
Visible spectrum	The visible spectra are comparable						
HPTLC profile	Main compound was detected						
Melting point °C	150	149.1	149.5	148.7	148.1	149	
Titre (g/100g) Potentiometry	97.3	96.0	99.1	99.9	98.5	99.9	99.4
HPLC content % (peak area)	>99.5	>99				>99.5	
Impurities (HPLC) g/100g							
Impurity A	<0.1, N.D.	<0.1, N.D.					
Impurities B &C	<0.1, D	<0.1 D					
Acetic acid (g/100g)	0.06	1.6					
K^+ content (g/100g)	0.02	0.8					
Isopropanol (µg/g)	150	<25, ND					
Ethanol (µg/g)	<100, D	<25, ND					
n-Propanol (µg/g)	3500	<50, D					

D: Detected, ND: Not detected
Impurity A: N-(4-amino-phenyl)-acetamide
Impurity B: 2-[(2-hydroxy-ethyl)-m-tolyl-amino]-ethanol
Impurity C: N-(4-{4-[bis-(2-hydroxy-ethyl)-amino]-2-methyl-phenylazo}-phenyl)-acetamide

3.1.5. Impurities / accompanying contaminants

In addition to the impurities mentioned in 3.14 and other impurities are as given below

Water content: ≤ 2 g/100g
Heavy Metals
- As, Sb, Hg: each < 5 mg/kg
- Cd: < 10 mg/kg
- Pb: < 20 mg/kg

Sulphated ash content: ≤ 2 g/100g

3.1.6. Solubility

- Water*: 35 mg/L at 20°C (according to 84/449/EEC method A6)

Solubility (g/100ml - 22°C after 24 h)

- Ethanol: 1 ≤ S ≤ 10

- DMSO: 1 ≤ S ≤ 10

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 2.59 at 24 °C (according to 84/449/EEC method A8)

3.1.8. Additional physical and chemical specifications

Melting point:	148 – 150 °C
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
UV_Vis spectrum (200-800 nm)	absorption maxima at 419.0 and 449.8 nm

3.1.9. Homogeneity and Stability

The analytical procedure was validated for the quantification of HC Yellow n° 7 (batch No. 0509831) over a range of 1 - 100 µg/mL. The limit of quantification was assessed at mg/mL in 0.5% CMC (carboxymethylcellulose) and 0.5% MC (methylcellulose) and at 0.001 mg/mL in DMSO and DMF.

The homogeneity of the test item at 10 and 200 mg/mL in 0.5% CMC (CV4-6%) and at 14 mg/mL in 0.5% MC (CV 3%) on the day of preparation was satisfactory.

The stability of the test item in dosage forms at 10 and 200 mg/mL in 0.5% CMC (deviation from nominal value: 2-9%), at 14 mg/mL in 0.5% MC (deviation from nominal value: 4%), at 0.1 and 250 mg/mL in DMSO (deviation from nominal value: 1-7%) and at 5 and 250 mg/mL in DMF (deviation from nominal value: 1-7%) was satisfactory over a 4-hour period at room temperature, protected from light and under inert gas atmosphere.

General comments to physico-chemical characterisation

- The stability of HC Yellow n° 7 in typical hair dye formulations has not been reported.

3.2. Function and uses

HC Yellow n° 7 is used in semi-permanent hair dye formulations at a maximum concentration of 0.25%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	OECD 401
Species/strain:	rat, Sprague-Dawley
Group size:	10 females (5 per doses)
Test substance:	HC Yellow n°7
Batch:	0509831
Purity:	97.3%
Vehicle:	0.5% carboxymethylcellulose
Dose:	50 and 500 mg/kg bw (10 mL/kg)
Route:	oral gavage
GLP statement:	in compliance
Study period:	November 2004 – January 2005

HC Yellow n° 7 was administered by oral intubation to female Sprague-Dawley rats at the doses of 50 and 500 mg/kg bw (5 females per dose). A preliminary test in which one female was given 500 mg/kg preceded the definitive test. During the observation period of 14 days, a record was kept for mortalities and signs of toxicity. Body weights were recorded on day 1 and then on days 8 and 15 for the surviving animals. All rats that died were investigated macroscopically to identify organ changes in the skull, thorax and abdomen and surviving animals were similarly examined at the end of the 14-day post-observation.

Results
In the preliminary test, hypoactivity, dyspnea and piloerection were observed from day 1 to day 3 in the animal given 500 mg/kg bw.

At 500 mg/kg bw, 3 of the 5 female rats died on day 2. Piloerection and orange-colored extremities were observed prior to death. Surviving animals showed piloerection on day 1 or until day 3 (together with dyspnea and reduce activity for the latter rat). No mortalities and no clinical signs were observed at 50 mg/kg bw.

No effect on the body weight was recorded in the surviving rats given 500 mg/kg bw. At 50 mg/kg bw, a reduced body weight gain was noted in 1/5 females during the first week of the study and in 1/5 females during the second week of the study when compared to historical control animals.

At necropsy, no apparent abnormalities were observed in any animal.

The results of the test indicated that the maximal non-lethal oral dose was 50 mg/kg in female rats and the minimal lethal dose was 500 mg/kg bw. 500 mg/kg bw is considered close to the median lethal dose (LD50).

HC Yellow n° 7 is considered to be moderately toxic following a single oral administration.
Ref.: 1

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

Guideline:	OECD 404
Species/strain:	New-Zealand white rabbits
Group size:	3 females
Test substance:	Imexine AA
Batch:	Op. 35
Purity:	99.1%
Vehicle:	neat
Dose level:	/
Dose volume:	500 mg
GLP:	yes
Study period:	27-30 July 1989

One day prior to the application of Imexine AA, the dorsal area of the trunk of each animal was clipped free of fur. A 500 mg sample of neat Imexine AA moistened with water was applied to the skin of each animal. It was held in contact with the skin for 4 hours by means of a semi-occlusive dressing. Subsequently, the dressings were removed, any residual test substance was wiped off and the treated area was observed 1, 24, 48 and 72 hours after removal of the dressing.

Results
There were no skin reactions. Brown/yellow skin discolouration was observed at the 1-hour time-point but did not prevent the accurate assessment of erythema.

Conclusion
Under the conditions of this study, Imexine AA was not irritating to rabbit skin when tested undiluted.

Ref.: 2

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405
Species/strain:	New-Zealand white rabbits
Group size:	3 females
Test substance:	Imexine AA
Batch:	Op 35
Purity:	99.1%
Vehicle:	neat
Dose level:	/
Dosing volume:	50 mg (eq. 0.1 ml)
GLP:	yes
Study period:	2-5 August 1989

A 50 mg sample of neat Imexine AA was instilled into the conjunctival sac of the left eye of the animals after gently pulling the lower lid away from the eye ball. The lids were then held together for about one second to avoid any loss of test substance, and the eyes were not rinsed after instillation. The untreated right eye served as control, and ocular reactions were assessed 1, 24, 48 and 72 hours after instillation.

Results
Conjunctival reactions consisted of slight to moderate redness and chemosis, mainly until 24 hours after instillation. There were no ocular reactions at the 72-hour observation, and the study was then terminated.

Conclusion
Under the conditions of this study, Imexine AA was slightly irritating to rabbit eyes when tested undiluted.

Ref.: 3

3.3.3. Skin sensitisation

Guideline: OECD 429
Species/strain: CBA/J mice
Group size: 28 female (7 groups of 4 mice)
Test substance: HC Yellow n° 7
Batch: 0509831
Purity: 97.3%
Vehicle: dimethylformamide (DMF)
Concentration: 1, 2.5, 5, 10 or 25% (w/v)
Positive control: α -hexylcinnamaldehyde at 25% (v/v) in DMF
GLP: yes
Study period: 1-6 December 2004

Animals were separated in 7 groups (4 mice/group) consisting of:
* 5 treated groups receiving HC Yellow n° 7 at 1, 2.5, 5, 10 or 25% (w/v) in dimethylformamide (DMF). This vehicle was selected on the basis of the results from a previous solubility study showing that HC Yellow n° 7 was non-soluble in other recommended vehicles, and that 25% (w/v) in DMF was the maximal practicable concentration. This concentration was non-irritating in a preliminary test.
* A negative control group receiving the vehicle (DMF) alone.
* A positive control group receiving α-hexylcinnamaldehyde (HCA) at 25% (v/v) in DMF.

The test substance HC Yellow n° 7, DMF or HCA was applied on the ears (25 µL per ear) of the animals for 3 consecutive days designated as days 1, 2 and 3. After 2 days of resting (day 6), mice received a single intravenous injection of tritiated methyl thymidine (^{3}H-TdR). Lymph nodes draining the application sites (auricular nodes) were sampled, pooled per group, and the proliferation of lymphocytes was evaluated by measuring the incorporation of ^{3}H-TdR. The values obtained were used to calculate stimulation indices (SI), and the EC_3 was estimated (theoretical concentration resulting in a SI of 3). The irritant potential of HC Yellow n° 7 was assessed by measuring ear thickness on days 1, 2, 3 and 6.

Results

HC Yellow n° 7 %	SI
1	0.87
2.5	0.77
5	1.21
10	1.41
25	1.44
α-hexylcinnamaldehyde 25% (v/v)	5.22

There were no irritation reactions attributed to HC Yellow n° 7. There were no lymphoproliferative responses, and the threshold SI of 3 values was not approached in any of the HC Yellow n° 7 groups.

Conclusion
Under the conditions of this murine Local Lymph Node Assay, HC Yellow n° 7 did not induce delayed contact hypersensitivity.

Ref.: 4

Comment
HC Yellow n° 7 is a non-sensitiser in the LLNA.

3.3.4. Dermal / percutaneous absorption

***In Vitro* percutaneous Absorption Study using human dermatomed Skin**

Guideline:	/
Tissue:	Human female abdominal skin from 4 donors
Group size:	8 diffusion cells in 2 separate experiments
Skin integrity:	Trans Epidermal Water Loss
Diffusion cell:	flow-through diffusion cells
Test substance:	Imexine AA
Batch:	0503175
Purity:	99.4%
Test item:	semi-permanent hair dye formulation containing 0.11% Imexine AA.
Dose volume:	20 mg/cm² (21.7 µg/cm² of Imexine AA).
Receptor fluid:	Ca and Mg-free phosphate-buffered saline containing 0.25% Tween 80
Solubility receptor fluid:	/
Stability receptor fluid:	/
Method of Analysis:	HPLC
GLP:	Yes
Study period:	17 October – 10 November 2000

Human abdominal skin samples were obtained from four different female donors subjected to plastic surgery. The skin samples were transferred at +4°C and kept frozen at -20°C until use.
Skin samples were dermatomed (593 ± 148 µm in thickness) and mounted in flow-through diffusion cells, using calcium and magnesium-free phosphate-buffered saline containing 0.25% Tween 80 as the receptor fluid. The integrity of the skin was checked by measuring Trans Epidermal Water Loss. Eight diffusion cells were used in two separate experiments, and skin was maintained at approximately 32°C.
A typical semi-permanent hair dye formulation containing 0.11% Imexine AA was applied to the skin surface at 20 mg/cm² (corresponding to exactly 21.7 µg/cm² of Imexine AA). This concentration was the maximum practicable concentration, given the low solubility of Imexine AA in hair dye formulations devoid of other dye ingredients. After 30 minutes, the remaining formulation on the skin surface was removed using a standardized washing procedure, simulating use conditions. Twenty-four hours after application, the percutaneous absorption of Imexine AA was estimated by measuring its concentration by HPLC and UV-visible detection in the following compartments: skin excess, *stratum corneum* (isolated by tape strippings), living epidermis/dermis and receptor fluid.

Results
Most of the Imexine AA applied on the skin surface was removed with the skin washes (skin excess, about 105% of the applied dose), and the total recovery rate was about 105%.
The mean amounts of Imexine AA considered as absorbed (dermal delivery) were estimated as follows (sum of the amounts measured in living epidermis/dermis and receptor fluid): 0.011 ± 0.008 µg/cm^2 (0.05 ± 0.03% of the applied dose).

Cutaneous Distribution	µg/cm²	% applied dose
Skin excess	22.82 ± 1.08	104.95 ± 0.37
Stratum corneum	0.023 ± 0.028	0.09 ± 0.13
Living epidermis/dermis	0.008 ± 0.004	0.04 ± 0.02
Receptor fluid	0.003 ± 0.003	0.01 ± 0.02
Dermal delivery*	**0.011 ± 0.008**	0.05 ± 0.03

* receptor fluid + living epidermis/dermis

Conclusion
The amounts of Imexine AA considered as absorbed from a typical semi-permanent hair colouring formulation containing Imexine AA at 0.11% were estimated to be 0.011 ± 0.008 µg/cm^2 (0.05 ± 0.03% of the applied dose).

Ref.: 14b

Comment
The experiment did not follow an accepted guideline. In addition, the concentration of Imexine AA was too low, which was due to the low solubility in the hair dye formulation used. Therefore, the mean + 2SD (0.011 + 2 x 0.008 or 0.027 µg/cm^2) may be used for calculating the MOS.

Normally, the absorption would have been adjusted to take account of the low concentration tested. However, in view of the very high MOS calculated from the mean + 2SD, any such adjustment in this particular case is unnecessary as it will not affect the safety assessment.

3.3.5. Repeated dose toxicity

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

No data submitted

3.3.5.2. Repeated Dose (13 weeks) oral / dermal / inhalation toxicity

Guideline: OECD 408
Species/strain: Sprague-Dawley rats
Group size: 10 male and 10 female rats per dose
Test substance: HC Yellow n° 7
Batch: Lot T 68
Purity: 99.9%
Dose levels: 0, 10, 40 and 160 mg/kg bw/d
Vehicle: 0.5% carboxymethylcellulose (5 mL/kg)
Route: oral
Administration: daily oral gavage for 13 weeks
GLP statement: in compliance
Study period: April 1994 – July 1994

In this 13-week oral Toxicity Study in Sprague-Dawley, HC Yellow n° 7 was administered by gavage at dose levels of 0, 10, 40 and 160 mg/kg bw/d to male and female rats (10 animals per dose). The dose levels were selected on the basis of the results from a 4-week oral toxicity study in rats performed at 25, 80 and 250 mg/kg bw/d (SCCNFP/0675/03, 2003). Clinical observations were recorded daily. Animals were weighed at the start of the study and weekly thereafter. Ophthalmological examinations were performed on all animals of each sex of the control and the high dose group before the beginning of the treatment period and on week 12. In addition, following abnormalities observed in the high dose group, all animals of the low and intermediate dose were examined on week 12. These examinations included corneal reflex and the examination of the appendages, optic media and fundus by indirect ophthalmoscopy. Food consumption per cage was determined weekly. Haematological, chemical chemistry investigations and urine analysis were

performed in week 13. Complete necropsies and histopathological exam were performed on all animals.

Results
All animals survived to the end of the studies.

Ptyalism was observed at 40 (1/10 males and 2/10 females) from week 10 and at 160 mg/kg bw/d (7/10 males and all females) from week 2. This clinical sign was considered to be treatment-related. Yellow/orange coloured urine, was observed in all treated animals, and yellow/orange coloured tail, body extremities or fur in some treated animals, mostly in a dose dependent way. Other clinical signs such as regurgitation, scabs on head or on back or scattered hair were not attributed to the test substance.

At ophthalmological examination, yellowish bilateral discolouration of the fundus was noted for 7/10 males given 40 mg/kg bw/d and in most animals at 160 mg/kg bw/d, which was attributed to the staining properties of HC Yellow n° 7. Other minor findings such as unilateral opacification of the lens or corneal vacuolization were observed in some rats. They were considered of no toxicological significance.

Slight reduced body weight gains were observed in females given 160 mg/kg bw/d, which resulted at the end of the study in a lower mean body weight when compared to the control rats (-7%). There were no changes in male body weight or in food intake in either sex.

Some slight differences from controls were noted in the haematological parameters including erythrocyte count, mean cell volume and mean cell haemoglobin in males and packed cell volume, mean cell volume and mean cell haemoglobin concentration in females were noted. They were considered to be of no toxicological significance since they were minor, often not dose-related and the individual values were within or close the normal range of background data.
The results of blood biochemistry showed slight differences between treated and control animals: slightly lower glucose in females given 40 and 160 mg/kg bw/d, mean higher blood sodium levels in females given 10 mg/kg bw/d and in males given 40 mg/kg bw/d and mean higher blood inorganic phosphorus levels in females given 40 and 160 mg/kg bw/d and a slight decrease in males given 10 mg/kg bw/d. Mean blood urea concentration was also slightly affected in females at 160 mg/kg bw/d as well as mean blood alkaline phosphatase. Mean blood albumin concentration was slightly increased in males at 160 mg/kg bw/d.
There were no changes in urinary parameters in rats of either sex.
When compared to controls, statistically significant higher mean absolute and relative liver and kidney weights were noted in both sexes at 160 mg/kg bw/d. Although they were not associated with relevant histopathological abnormalities, it was considered to be treatment-related. The higher mean kidney weight, which is possibly correlated to the higher incidence and severity of tubular basophilia in the treated animals given 40 and 160 mg/kg bw/d, was attributed to the administration of the test substance.
There were no macroscopic findings attributed to treatment with HC Yellow n° 7 apart from yellow/orange discolouration of some tissues in animals given 160 mg/kg/d (mesentery, adipose tissue).
At microscopic examination, brownish pigment deposits in thyroid follicular epithelial cells were observed for some males and females given 160 mg/kg bw/d. These pigment deposits were well tolerated by the surrounding tissue as there were no associated inflammatory, degenerative or proliferative changes. They were considered to be of minor toxicological importance.
When compared to controls, a higher incidence and/or severity of tubular basophilia was observed in both sexes at 40 and 160 mg/kg/d. These changes were sometimes associated with thickening of the tubular basal membrane. Although tubular basophilia is commonly found spontaneously in the untreated laboratory rat of this strain and age, its higher

incidence in the animals given 40 or 160 mg/kg bw/d was considered to be treatment-related.
No histopathlogical abnormalities were found in the adipose tissue and mesentery, which showed colouration at macroscopic examination.
Other findings or minor changes were observed in some treated animals but as they are commonly recorded spontaneously in this strain of rats, were considered not to be of toxicological importance.

Conclusion
The administration of HC Yellow n° 7 daily by gavage to rats for 13 weeks did not produce any signs of toxicity at 10 mg/kg/d. The target organs were likely the liver and kidneys. The main changes observed at 40 and 160 mg/kg/d were kidney microscopic findings (tubular basophilia), associated at 160 mg/kg/d with slightly higher kidney weights relative to controls (both sexes) and a slight increase in blood urea concentrations (females).
Accordingly, under the conditions of this study, the No Observed Adverse Effect Level (NOAEL) was 10 mg/kg bw/d.

Ref.: 5

3.3.5.3. Chronic (> 12 months) toxicity

No Data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Taken from SCCNFP/0675/03

Bacterial Reverse Mutation Test

Guideline:	OECD 471 (1983)
Species/strain:	*S. typhimurium,* TA98, TA100, TA1535, TA1537, *E. coli* WP2 uvr A
Replicates:	Triplicate plates, 2 independent tests
Test substance:	IMEXINE AA dissolved in DMSO
Batch:	Op. T 68
Purity:	99.9 %
Concentrations:	156.25-2500 µg/plate, with and without metabolic activation
GLP:	In compliance
Study period:	14 April – 13 May 1994

HC Yellow n° 7 has been investigated for gene mutation in *S. typhimurium* and *E. coli* using the plate incorporation method. Liver S9 fraction from Aroclor 1254 treated rats was used as the exogenous metabolic activation system. Negative and positive controls were in accordance with the OECD guideline. The concentration range 156.25-2500 µg/plate was selected on the basis of a preliminary toxicity indicating that 2500 µg/plate was cytotoxic and at the limit of solubility.

Results
The substance induced a concentration-related increase in numbers of revertants in TA1537 and TA98 with metabolic activation. There were no significant increases with other strains or in the absence of S9-mix. The negative and positive control agents gave the expected results. The compound is considered mutagenic in this system.

Ref.: 6

Comment of the SCCS
The second test with S9-mix was performed as a preincubation test. The test compound was clearly positive in the frameshift strain TA1537 and TA98 with S9-mix in both performed test. The maximum increase in revertants compared to controls were 5.4 and 4.9 in TA1537 and 6.6 and 25.5 in TA98 in the first and second test respectively

Taken from SCCNFP/0675/03

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

Guideline:	OECD 476 (1984)
Cells:	L5178Y (TK+/-) mouse lymphoma cells
Replicates:	2 independent tests
Test substance:	IMEXINE AA in DMSO solution
Batch:	Op. T 68
Purity:	99.9 %
Concentrations:	37.5-600 µg/ml, with and without metabolic activation
GLP:	In compliance
Study period:	15 April – 18 May 1994

HC Yellow n° 7 has been investigated for gene mutation at the TK locus in L5178Y (TK+/-) mouse lymphoma cells. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. The concentration range 37.5-600 µg/ml was selected on the basis of a preliminary toxicity indicating that concentrations of 1000 and 5000 µg/ml were cytotoxic. Negative and positive controls were in accordance with the OECD guideline.

Results
In both experiments the substance induced a concentration-related increase in mutation frequency in the absence of S9-mix. In the presence of S9-mix, an increase was seen only at 300 µg/ml, which was associated with severe toxicity. No cells survived at higher concentrations. The negative and positive control agents gave the expected results. The compound is considered mutagenic in this system.

Ref.: 7

Comment of the SCCS
There was a clear and concentration related increase without S9-mix in both performed assays with 6.5 and 5.1 fold increase at the highest concentrations tested, which was close to the increase in positive controls (7.9 and 7.3 respectively). The number of induced mutants in the two assays without S9-mix was 268 and 362 respectively. In the first assay with S9-mix, there was a clear and concentration related increase up to 13 fold increase in relative mutation frequency and an increase of 694 in absolute mutants. For the positive control this values were 5.1 and 310, which is just above the acceptable value of 300. In the second experiment with S9-mix there was also a concentration related increase in the absolute (174) and relative (4.5) mutant frequency. In this assay the response to positive control was low: increase in absolute mutants of 170 and in relative mutation frequency of 2.9.

Submission III, 2005

Guideline:	OECD 476
Species/strain:	L5178Y mouse lymphoma cells (hprt-locus)
Test substance:	HC Yellow n° 7
Batch:	0509831
Purity:	97.3%

Vehicle:	DMSO
Concentrations:	exp. 1: 0, 25, 50, 100, 125, 150, 175 µg/ml without S9-mix 0, 25, 50, 100, 125, 150, 175, 200 µg/ml with S9-mix Exp. 2: 25, 50, 75, 100, 125, 150, 175, 200, 225, 250 µg/ml (without and with S9-mix) Exp. 3: 50, 100, 125, 150, 175, 200, 225, 250 µg/ml (with S9-mix)
Control:	4-nitroquinoline 1-oxide (without S9-mix) benzo[a]pyrene (with S9-mix)
GLP:	in compliance
Study period:	26 July – 31 October 2004

- The test item HC Yellow n° 7 was first evaluated in two independent experiments using duplicate cultures each (single cultures for positive controls). Both experiments used a pulse (3-hour) treatment and were conducted in the absence (-S9-mix) and presence (+S9-mix) of metabolic activation (S9-mix prepared from the livers of rats given Aroclor 1254). In order to clarify the results obtained in the second experiment, a third experiment was performed in the presence of metabolic activation. On the basis of a cytotoxicity range-finder experiment, HC Yellow n° 7 was tested in the first two experiments at concentrations up to 300 µg/mL. A range of concentrations was selected for final test statistics as given above.

Known mutagens in the presence (Benzo(a)pyrene, BP) or absence of S9-mix (4-nitroquinoline 1-oxide, NQO) were tested at two different concentrations and served as positive controls. Negative controls consisted of cultures treated with the solvent alone (DMSO). Cells were suspended in culture medium and exposed to various concentrations of the test item, to solvent or positive controls. After the treatment period (3 hours), the cells were resuspended in culture medium. They were transferred to flasks for growth through the expression period (7 days) or were diluted to be plated for survival (7 to 8 days). At the end of the expression period, acceptable cultures were then plated for viability (2 plates per culture, 7 days) or 6-TG resistance (4 plates per culture, 11-12 days).

Results
Mutation frequencies in solvent negative controls remained within normal ranges, and treatment with positive controls NQO and BP yielded distinct increases in mutant frequency. Accordingly, the study was considered to be valid.
In the absence of S9-mix, no statistically significant increases in mutant frequency were observed in either experiment.
There were no increases in mutant frequency in the presence of S9-mix in the first experiment. In the second experiment in the presence of S9-mix, slight but statistically significant increases in mutant frequency were observed at the three highest concentrations tested (200, 225 and 250 µg/mL). Such increases were not reproduced in the third, confirmatory experiment. As the increases observed in the presence of S9-mix were not reproduced in two out of three experiments, and as they were primarily due to isolated increases in only one out of the two replicate cultures, they were considered to be of no biological significance. Moreover, there was no dose-relationship over the highest concentrations tested in experiment 2. Finally, the mutant frequencies obtained at the two highest concentrations tested (225 and 250 µg/mL) remained within the laboratory historical solvent control range. The upper limit of this historical control range (calculated as mean mutant frequency + 2 standard deviations) was only marginally exceeded at the concentration of 200 µg/mL (16.3 *versus* 14.4 mutants per 10^6 viable cells), and such minimally high mutant frequency values can be spontaneously encountered in solvent control cultures.

Conclusion
It was concluded that HC Yellow n° 7 did not induce gene mutations at the hprt locus in the absence or presence of S9-mix.

Ref.: 8

***In vitro* Mammalian Erythrocytes Micronucleus Test**

Guideline:	draft OECD 487
Species/strain:	Human peripheral blood lymphocytes
Replicates:	Three experiments with duplicate cultures
Test substance:	HC Yellow n° 7
Batch:	0509831
Purity:	97.3%
Vehicle:	DMSO
Concentration:	Exp. 1: 25, 14, 180 µg/ml (without S9-mix) 200, 400, 450 µg/ml (with S9-mix) Exp. 2: 50, 140, 180 µg/ml (without S9-mix) 200, 425, 500 µg/ml (with S9-mix) Exp. 3: 100, 110, 120, 130, 140, 150 µg/ml (without S9-mix)
Control:	without S9-mix: vinblastine (VIN), 4-nitroquinoline 1-oxide (NQO) With S9-mix: cyclophosphamide (CPA)
GLP:	in compliance
Study period:	28 July 2004 – 14 February 2005

The test item HC Yellow n° 7 was evaluated in two independent experiments in the absence and presence of metabolic activation (S9-mix prepared from the livers of Aroclor 1254-treated rats). In order to clarify the results obtained in the first experiment, a third experiment was performed in the absence of metabolic activation. The top concentration in each experiment and test condition was selected to produce approximately 60% cytotoxicity (reduction in replication index, RRI), and a range of concentrations covering low to marked cytotoxicity was selected for micronucleus analysis as given above.

Duplicate cultures were treated with each concentration of HC Yellow n° 7 or with known clastogens in the presence (cyclophosphamide, CPA) or absence of S9-mix (4-nitroquinoline-1-oxide, NQO and vinblastine, VIN). Solvent-treated cultures (DMSO, four replicates) were used as negative controls.
In experiment 1, cultures were incubated in the presence of the mitogen phytohaemagglutinin (PHA) for 24 hours and then received a 20- or 3-hour treatment in the absence or presence of S9-mix, respectively. Cells were harvested 72 hours after the beginning of incubation (the last 28 hours of incubation being in the presence of cytochalasin B). In experiment 2, a similar test procedure was used, except that cultures were incubated in the presence of PHA for 48 hours prior to treatment (harvesting took place 96 hours after the beginning of incubation). The test procedure used in the confirmatory experiment 3 was similar to that used in experiment 1 in the absence of metabolic activation (24-hour mitogen stimulation prior to treatment).
Lymphocyte preparations were stained and examined microscopically for determining the replication index and the proportion of micronucleated binucleate (MNBN) cells when selected. Two thousand binucleate cells per concentration were analysed blind.

In the presence of S9-mix, treatment of cultures with HC Yellow n° 7 did not produce any significant increase in MNBN cell frequency in either test condition (24 or 48 hours PHA stimulation prior to treatment). Similarly, there were no increased MNBN cell frequencies in the absence of metabolic activation where treatment commenced 48 hours after PHA stimulation (experiment 2).
In the absence of S9-mix, treatment of cultures with HC Yellow n° 7 was associated with statistically significant increases in MNBN cell frequency in both experiments where treatment commenced 24 hours following PHA stimulation (experiments 1 and 3).
These increases were observed in experiment 1 at the highest concentration of 180 µg/mL associated with severe cytotoxicity (65% reduction in RI), and in experiment 3 at concentrations of 100 µg/mL and higher that were most of the time associated with

moderate cytotoxicity. Since these increases were reproduced in two independent experiments, they were considered to be evidence of a weak positive response.

Conclusion
Under the conditions of this study, HC Yellow n° 7 produced an increase in the number of human lymphocytes with micronuclei in the absence of metabolic activation, in the experiments where treatment commenced 24 hours after mitogen stimulation.

Ref.: 9

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

A mammalian erythrocyte micronucleus test (Ref. 10, Subm. II) was described in opinion SCCNFP/0675/03. This study was considered not acceptable due to methodological shortcomings and thus is not included here.

Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474
Species/strain:	Mice, Swiss OF1
Group size:	10 animals (5 males and 5 females) per group
Test substance:	IMEXINE AA
Batch:	No. opT77
Purity:	98.5%
Vehicle:	0.5% aqueous methylcellulose
Dose levels:	0, 35, 70, 140 mg/kg bw/d, twice at 24 hours interval
Sacrifice times:	24 hours
GLP:	in compliance

Groups of 10 mice (5/sex/dose level + 3 spare mice/sex at the highest dose level) received two oral (gavage) doses of HC Yellow n° 7, 24 hours apart, at 0, 35, 75 or 140 mg/kg bw/d in 0.5% aqueous methylcellulose (10 mL/kg). These dose levels were selected on the basis of the results from a sighting test where deaths were observed at 200 mg/kg bw/d and higher. Animals were killed 24 hours after the last dosing. An additional positive control group of 5 mice/sex was given a single oral dose of cyclophosphamide (CPA) at 50 mg/kg, and mice were killed 24 hours after dosing.
For each animal, smears were prepared from the femoral bone marrow, stained with Giemsa and scored blind for the incidence of micro-nucleated polychromatic erythrocytes (MN-PCE, 2000 PCE counted) and for the polychromatic/normochromatic erythrocyte ratio (PCE/NCE ratio, 1000 erythrocytes counted).

Results
When compared to controls, the incidence of MN-PCE was statistically significantly increased in animals given the positive control CPA (approximately 17-fold the mean concurrent control value), showing the adequate sensitivity of the test system and procedure used.
There were no deaths or clinical signs following treatment with HC Yellow n° 7 up to 140 mg/kg bw/d.
There were no increases in the incidence of MN-PCE in any group treated with HC Yellow n° 7. Finally, there was no indication of bone marrow toxicity since PCE/NCE ratios were similar for HC Yellow n° 7 and control groups.

Conclusion
Under the conditions of the study, HC Yellow n° 7 did not induce cytogenetic damage leading to micronucleus formation in the bone marrow of mice treated orally up to 140 mg/kg bw/d, the Maximum Tolerated Dose.

Ref.: 10

Comment
Although there were no bone marrow toxic effects in this study, the oral bioavailability of HC Yellow n° 7 in mice was confirmed in a plasma pharmacokinetic study where high systemic exposure was observed following dosing at 140 mg/kg bw/d.

Taken from SCCNFP/0675/03

Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo*

Guideline:	OECD 475, draft (1991)
Species/strain:	Wistar rat, HanIbm: WIST (SPF) strain
Group size:	4 males
Test substance:	IMEXINE AA in PEG 300
Batch:	Op. T 68
Purity:	/
Dose levels:	25 and 250 mg/kg bw, by gavage
Sacrifice times:	16 hours, all dose groups; 2 hours, high dose group
GLP:	In compliance

HC Yellow n° 7 has been investigated for induction of unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro* following *in vivo* dosing. A preliminary toxicity study indicated that 250 mg/kg bw was close to the MTD and therefore this was used as the highest dose. Negative and positive controls were in accordance with the OECD guideline.
Animals were sacrificed after 16 hours and for an additional high dose group after 2 hours.
Hepatocytes were isolated and at least 3 cultures were established per animal. The hepatocytes were subsequently treated with 3H-thymidine *in vitro* for 4 hours. Incorporation of radio-label was assessed using auto-radiography.

Results
One animal died within 16 hours of dosing at 250 mg/kg bw; the cause of death was not established. There were no differences in the viability of hepatocytes isolated from rats of different dose groups. The results met all the pre-defined criteria for a negative response and therefore the test substance was not found to induce UDS. The positive control agent gave the expected results.

Ref.: 11

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Prenatal developmental study

Guideline:	OECD 414 (2001)
Species/strain:	rat, Sprague-Dawley
Group size:	100 (25 presumed-pregnant females per group)
Test substance:	HC Yellow n° 7
Batch:	op.26

Purity:	99.9%
Vehicle:	0.5% carboxymethylcellulose
Dose levels:	0, 25, 80, and 240 mg/kg bw/d
Dose volume:	5 ml/kg bw
Route:	oral, gavage
Administration:	once daily on Day 6 through Day 15
GLP statement:	in compliance
Study period:	December 1990 – January 1991

In this oral developmental toxicity study, HC Yellow n° 7 was administered orally once daily to 100 presumed-pregnant Sprague-Dawley rats from day 6 to day 15 of pregnancy at the doses of 0, 25, 80 and 240 mg/kg bw/d in 0.5% carboxymethylcellulose (25 rats per dose). The dosage volume was 5 ml/kg bw.

Viabilities, clinical observations, body weights and feed consumption values were recorded. All surviving rats were sacrificed on day 20 and subjected to macroscopic examination. Usual litter parameters were recorded and foetuses were sexed, weighted and submitted to external examination. For the first 20 litters per group, approximately half of the live foetuses were examined for visceral anomalies, and the remaining foetuses were examined for skeletal anomalies.

Results

Maternal parameters:

One female rat in the 240 mg/kg/d dosage group which presented some clinical signs on day 9 of pregnancy (piloerection, paleness, ataxia and yellowish ocular discharge) was sacrificed. Neither deaths nor abortions were observed in the other rats of either group.

Orange coloured urine was observed in all of the groups treated with HC Yellow n° 7.

A slight body weight loss was observed in the rats treated at the dose of 240 mg/kg bw/d on the first 3 days of treatment and the body weight gain was consequently lower than that of the control animals during the treatment period. In the 80 mg/kg bw/d group, the body weight gain of females with completed pregnancy was slightly lower than that of control during the treatment period. In the 25 and 80 mg/kg bw/d treated rats, food consumption was comparable to that of the control animals. In the 240 mg/kg bw/d group, food consumption was reduced by about 25% when compared to that of the control group during the treatment period.

No significant differences were observed in the number of *corpora lutea* in the 80 and 240 mg/kg bw/d treated groups when comparing with the control rats. No significant differences in the number of implantation sites were observed in the 80 and 240 mg/kg bw/d treated groups comparing with the control rats. In the 25 mg/kg bw/d group, the number of *corpora lutea* and implantation sites were slightly lower than that of the control group. This reduction was without any relationship with the treatment, as the dosing of dams began after implantation of the *concepti*.

Foetal parameters:

The mean number and rate of live foetuses was similar in the control, 80 and 240 mg/kg bw/d groups. In the 25 mg/kg bw/d group, the mean number of live foetuses was slightly lower than that of the control group as a consequence of the reduction in the number of implantation sites but the rate of live foetuses was similar to that of the control animals.

The sex ratio was comparable in all groups, except in the 80 mg/kg bw/d group where the percentage of male was lower as compared to the control group but it was not dose-related.

No significant differences in body weight were observed between treated and control animals. No treatment related foetal abnormalities were observed in the control, 25 and 80 mg/kg bw/d. In the 240 mg/kg bw/d, a slightly delayed skeletal development (increase in reduced ossification of sternebre) was observed. It was considered as a consequence to the moderate maternotoxicity observed at this dose level.

Conclusion
Neither embryotoxicity nor teratogenic effects were observed with HC Yellow n° 7 at any dose level. Based on the results of this embryo-foetal development toxicity study, the maternal NOAEL was 25 mg/kg bw/d and the developmental NOAEL was 80 mg/kg bw/d.

Ref.: 12

3.3.9. Toxicokinetics

Toxicokinetics after oral administration

Guideline:	/
Species/strain:	Mice, OF1
Group size:	24 males, 24 females (7 to 8 weeks old)
Test substance:	HC Yellow n° 7
Batch:	0509831
Purity:	97.3%
Vehicle:	0.5% methylcellulose
Dose levels:	140 mg/kg bw
Dose volume:	10 ml/kg bw
Route:	oral, gavage
Administration:	once
GLP:	in compliance except for the pharmacokinetic modelling
Study period:	November 2004

The experimental conditions used (animal species, strain and age, dose-level, route, vehicle and dosage volume) were similar to those used in the mouse micronucleus test to confirm that mice were systematically exposed to the test item. A single dose of the test substance (140 mg/kg bw; gavage) was applied to 24 male and 24 female mice. Blood samples were collected at 0.25, 0.5, 1, 2, 4, 8, 24, and 48 hours post-gavage. The plasma was analysed for test item levels by a validated HPLC/UV method.

Results
There were no deaths and no clinical signs. From around two hours after dosing, all animals had orange-coloured urine, suggesting renal elimination of the absorbed test item.

The study showed that mice were systematically exposed to HC Yellow n° 7 until 24 or 8 hours post-dosing for males and females respectively. The test substance was rapidly absorbed: plasma C_{max} (23.1 and 27.5 µg/mL for males and females respectively) was reached after 0.25 h; however for males, a second C_{max} (24.8 µg/mL) was noted at 1 hour post-gavage. Total exposure was 95.72 µg/mL*h for males and 65.46 µg/mL*h for females; $t1_{/2}$ was 7.85h for males and 5.54h for females. There were no major gender differences in the pharmacokinetic profile and parameters.

Conclusion
This study showed good bioavailability of HC Yellow n° 7 following oral administration to mice.

Ref.: 13

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

HC Yellow n° 7

(Non-oxidative conditions)

Absorption through the skin	**A** (mean + 2SD)	**=**	**0.027 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.015 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose	**SAS x A x 0.001/60**	**=**	**0.0003 mg/kg bw/d**
No observed adverse effect level (13 week, oral, rat)	**NOAEL**	**=**	**10 mg/kg bw/d**

Margin of Safety	**NOAEL / SED**	**=**	**38000**

3.3.14. Discussion

Physico-chemical properties
HC Yellow n° 7 is used in semi-permanent hair dye formulations at a maximum concentration of 0.25%. The stability of HC Yellow n° 7 in typical hair dye formulations was not reported.

Toxicity
HC Yellow n° 7 is considered to be moderately toxic following a single oral administration and 500 mg/kg is considered close to the median lethal dose in female rats.
In an oral 13 weeks study in rats, the No Observed Adverse Effect Level (NOAEL) was 10 mg/kg/d. The adverse effects observed in this study were mostly related to a slight renal toxicity.

In a rat teratogenicity study by oral route, neither embryotoxicity nor teratogenic effects were observed with HC Yellow n° 7 at any dose level up to 240 mg/kg bw/d. The maternal NOAEL was 25 mg/kg bw/d and the developmental NOAEL 80 mg/kg bw/d. The only adverse effects observed at 240 mg/kg bw/d were a slight body weight loss and reduction in food consumption in the pregnant rats.
No data on two-generation reproductive toxicity was submitted.
A toxicokinetic study of HC Yellow n° 7 after oral administration to mice showed oral biovailability.
The NOAEL of 10 mg/kg bw/d from the 13 week oral study in rats may be used for the calculation of the MOS.

Skin/eye irritation and sensitisation
Under the test conditions, HC Yellow n° 7 was not irritating to rabbit skin when tested undiluted. It was slightly irritating to rabbit eyes when tested undiluted.
HC Yellow n° 7 is a non-sensitiser in the LLNA.

Percutaneous absorption
As the experiment did not follow an accepted guideline, and the concentration of HC Yellow n° 7 applied was too low, the amounts absorbed should be considered to include 2SD. Therefore, 0.027 µg/cm² (0.011 + 2 x 0.008 µg/cm²) may be used for calculating the MoS.
Normally, the absorption would have been adjusted to take account of the low concentration tested. However, in view of the very high MOS calculated from the mean + 2SD, any such adjustment in this particular case is unnecessary as it will not affect the safety assessment.

Mutagenicity/genotoxicity
HC Yellow n° 7 was tested for all three genetic endpoints: gene mutations, structural and numerical chromosomal aberrations. The test compound induced frameshift mutations in bacteria both with and without metabolic activation. It induced gene mutations and/or chromosomal aberrations in mammalian (mouse lymphoma) cells at the *tk* locus (MLA/*tk* assay), mainly in the absence of metabolic activation. However it did not produce gene mutations at the *hprt* locus either in the absence or presence of metabolic activation when tested up to the limit of cytotoxicity indicating that HC Yellow n° 7 did not produce gene mutations in mammalian cells. In the *in vitro* micronucleus test, HC Yellow n° 7 produced increases in the frequency of micronucleated binucleate cells in the absence of metabolic activation. These results were consistent with those obtained in the MLA/*tk* assay. Taking into account the negative results on the *hprt* locus and the positive results in the *in vitro* micronucleus test, the positive response in the MLA/*tk* assay was most likely due to the *in vitro* clastogenic activity of HC Yellow n° 7.
HC Yellow n° 7 was non-genotoxic when tested *in vivo* in an oral mouse bone marrow micronucleus study conducted up to the Maximum Tolerated Dose (140 mg/kg bw/d). Though there were no bone marrow toxic effects in this study, the oral bioavailability of HC Yellow n° 7 in mice was confirmed in a plasma pharmacokinetic study where high systemic exposure was observed following dosing at 140 mg/kg bw/d.
Finally, HC Yellow n° 7 did not cause DNA damage leading to unscheduled DNA synthesis (UDS) in hepatocytes derived from rats treated orally up to the lethal dose of 250 mg/kg bw.
As the genotoxic effects found *in vitro* were not confirmed in *in vivo* tests, HC Yellow n° 7 can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No data submitted

4. CONCLUSION

Based on the data provided, the SCCS is of the opinion that the use of HC Yellow n° 7 as a non-oxidative hair dye with a maximum on-head concentration of 0.25 % does not pose a risk to the health of the consumer.

5. MINORITY OPINION

Not applicable

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