EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

HC Yellow n° 10

COLIPA n° B81

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

The SCCP adopted this opinion at its 12th plenary meeting on 19 June 2007

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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Opinion to be cited as: Opinion of the SCCP on HC Yellow n° 10, 19 June 2007

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1. BACKGROUND

Submission I for HC Yellow No. 10 with the chemical name 1,5-Di-(ß-hydroxyethylamino)-2-nitro-4-chlorobenzene was submitted to the Scientific Committee on Cosmetology (SCC) in June 1995 by COLIPA[1].

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted at its plenary meeting on 20 May 1998 an opinion (XXIV/1293/97) with the final conclusion that:

"*HC yellow n°10 has an acute oral toxicity of >5000 mg/kg bw in the rat. The substance can be classified as very slightly irritating to the eyes and slightly irritating to the skin. Percutaneous absorption of a formulation was 0.024 % in absence and 0.025 % in presence of hair. In a 28-day study with rats, 125 mg/kg/day is considered to be the No Toxic Effect Level. In the teratogenicity study, no signs of maternal or foetal toxicity were observed after administration of 500 mg/kg bw. It should be noted that the NOEL stems from an daily exposure for 28 days, whereas human exposure to semi-permanent hair dye is unlikely to be more frequent than twice a month. The compound was found to be not mutagenic.*"

The substance is currently regulated by the Cosmetics Directive (76/768/EC), Annex III, Part 2 under entry 24 on the List of substances, provisionally allowed, which cosmetic products must not contain except subject to restrictions and conditions laid down.

Submission II of HC Yellow No. 10 was submitted by COLIPA in July 2005. According to this submission HC Yellow No. 10 is used in semi-permanent (non-oxidative) hair colouring products at a general on-head concentration of 0.1%.

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 HC Yellow No. 10 safe for use in non-oxidative hair dye formulations with a on-head concentration of maximum 0.1 % in the finish product taken into account the scientific data provided?*

2. *Does the SCCP recommend any further restrictions with regard to the use of HC Yellow No. 10 in non-oxidative hair dye formulations (e.g. max conc. in the finish cosmetic product, dilution ratio with hydrogen peroxide, warning)?*

[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

HC Yellow n°10 (INCI)

3.1.1.2. Chemical names

1,5-di-(β-hydroxyethylamino)-2-nitro-4-chloro-benzene
2,2'-[(4-chloro-6-nitro-1,3-phenylene)-diimino]-bis-ethanol
2-[2-chloro-5-(2-hydroxy-ethylamino)-4-nitro-phenylamino]-ethanol
1-Chloro-2,4-(β-hydroxyethylamino)-5-nitro-benzene

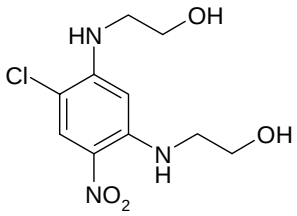
3.1.1.3. Trade names and abbreviations

Imexine FAH
COLIPA n° B81

3.1.1.4. CAS / EINECS number

CAS: 109023-83-8
ELINCS: 416-940-3 (Imexine FAH)

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Formula: $C_{10}H_{14}N_3O_4$ Cl

3.1.2. Physical form

Orange crystalline powder

3.1.3. Molecular weight

Molecular weight: 275.69

3.1.4. Purity, composition and substance codes

Material used in the market

Purity by UV assay: > 99 % (w/w)
Purity by HPLC assay: > 99.5 % (area)
Water content: < 0.1 %
Ash (w/w): <0.1 (w/w)

	Op. T8	Op. T10	0508386
Appearance	**Orange crystalline powder**		
Titre by spectro-photometry (g/100g)	> 99	> 99	> 99
Water content (g/100g)	< 0.1		0.01
Melting point (°C)	171 - 173	170.6	171
HPLC profile (purity) %	> 99.5		> 99.5
Impurity content (g/100g) (HPLC)			
- 1,2,4-trichloro-5-nitrobenzene	ND < 0.01		ND < 0.01
HPTLC Profile	In accordance with the specification		
Residual solvents (µg/g) (GC)			
- Ethanol	D < 500		D < 1000
Visible spectrum	The visible spectra are comparable		
Infra-Red spectrum	In accordance with the proposed structure		In accordance with the proposed structure
^{1}H and ^{13}C NMR spectra	In accordance with the proposed structure		In accordance with the proposed structure
Mass spectrometry	Compatible with the proposed structure		Compatible with the proposed structure

3.1.5. Impurities / accompanying contaminants

1,2,4-trichloro-5-nitro-benzene: < 0.01g/100g
Heavy metals
Hg, Sb, As: < 5 ppm (each)
Pb: < 20 ppm
Cd: < 10 ppm
Solvent (ethanol)*: contains 35 µg/0.1 g (350 ppm)
Chloride ions*: <0.001 mEq/g

* taken from opinion n° XXIV/1293/97

3.1.6. Solubility

in water: 79 mg/l (according to EEC method A6)
in ethanol: < 1 g/l at 22 °C
in DMSO: > 10 g/l at 22 °C

3.1.7. Partition coefficient (Log P_{ow})

Log P_{ow}: 1.38 at 23°C (EEC method A8)

3.1.8. Additional physical and chemical specifications

Melting point: 171-173 °C
Boiling point: /

Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
pH:	/
UV_Vis spectrum	λmax 388.2 nm

3.1.9. Homogeneity and Stability

The analytical procedure was validated for the quantification of HC Yellow n° 10 (B081) (batch n° 0508386) over a range of 5-100 µg/ml. The limit of quantification was assessed at 0.05 mg/ml in 0.5% methylcellulose (MC) and DMF and at 0.01 mg/ml in DMSO. The homogeneity of the test item at 10 and 200 mg/ml in 0.5% MC after day 0 and day 9 was satisfactory. The stability of the test item in the dosage forms at 10 and 200 mg/ml in 0.5% MC was satisfactory over a 6-hour period at room temperature and a 9-day period at +4°C, protected from light and under inert gas atmosphere. The stability of the test item in dosage forms at 0.1 and 250 mg/ml in DMSO and at 5, 10 and 100 mg/ml in DMF was satisfactory over a 4-hour period at room temperature, protected from light and under inert gas atmosphere.

General Comments to physico-chemical characterisation

- HC Yellow n° 10 is a secondary amine, and thus is prone to nitrosation. It should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb.
- No data was provided on the stability of HC Yellow n° 10 in marketed products.

3.2. Function and uses

HC Yellow n° 10 is used in semi-permanent (direct) hair colouring products at a general on-head concentration of 0.1%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Guideline:	OECD 420 (2001)
Species/strain:	Rat, Female, Sprague-Dawley Rj: SD (IOPS Han)
Group size:	5, (1 sighting test)
Test substance:	HC Yellow 10
Batch:	0508386
Purity:	99.8%
Dose:	2000 mg/kg
Vehicle:	0.5% methylcellulose in purified water
Route:	Gavage, 10 ml/kg
Observation:	14 days

GLP: in compliance

A sighting test was performed at 2000 mg/kg in one animal. It survived, but was hypoactive exhibited piloerection, dyspnoea and hypersalivation on day 1.
Thus 4 female rats at 2000 mg/kg were used for the main study. Animals were observed at least once daily for mortality/morbidity and daily for clinical signs over a period of 14 days following a single administration of the test substance. Body weights were recorded on day 1 prior to treatment, and on days 8 and 15 thereafter. Individual weights of animals found dead were measured at necropsy when survival exceeded 24 hours and if no signs of "cannibalism" were present. All study animals were subjected to a macroscopic examination as soon as possible after death.

Results
No deaths occurred. Clinical signs included hypoactivity, piloerection, and dyspnoea between 4 - 5 hours of dosing. A slightly reduced body weight gain was seen in 2/5 females during the second week of the study. The overall body weight gain of the other animals was similar to that of historical control animals. No abnormalities were observed at post mortem.

Conclusion
Under the conditions of this study, the maximum non-lethal dose of HC Yellow n° 10 following single oral gavage to fasted rats was 2000 mg/kg.

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: male New Zealand White rabbits
Group size: 3
Test substance: HC Yellow N°10
Batch: 0508386
Purity: 99.8%
Concentration: 1% of HC Yellow n° 10
Vehicle: 0.5% methylcellulose in purified water
GLP: in compliance

Test material 0.5% was applied on the skin using a patch (semi-occlusive dressing), to shaved intact skin on one flank of each rabbit. The first rabbit was treated for periods of 3 minutes, 1 hour and 4 hours. Since the dosage form was not severely irritant on this first animal, it was then applied for 4 hours to two other animals. Patch was removed and the site washed with water. The animals were examined 1, 24, 48 and 72 hours after patch removal. Adjacent areas of the treated skin of each animal served as controls.

Results
After a 3-minute or 1-hour exposure (one animal) no cutaneous reactions were noted. After a 4-hours exposure (three animals) a very slight erythema (grade 1) was noted in 2/3 animals on day 1 only. A yellow colouration of the skin, which could have masked a very

slight erythema (1/3 animals), was noted in all animals on day 1. None of the treated animals by HC Yellow N°10 showed that any erythema and/or oedema was observed after 4 hours exposure over 24, 48 and 72 hours.

Conclusion
Under the conditions of the experiment, HC Yellow N°10 at 1% in 0.5 % methylcellulose was non-irritant to the rabbit skin following a single dermal application.

Ref.: 2

3.3.2.2. Mucous membrane irritation

Guideline:	OECD 405
Species/strain:	male New Zealand White rabbits
Group size:	3
Test substance:	HC Yellow N°10
Batch:	N° 0508386
Purity:	99.8%
Vehicle:	1% of HC Yellow N°10 in 0.5% of methylcellulose in purified water
GLP:	in compliance

An 0.1 ml test preparation of HC Yellow N°10 at 1% (0.5% suspension of methylcellulose in purified water) was instilled into one eye (left conjunctival sac) of one the rabbits. The right eye was not treated and served as a control. Observations were made 1, 24, 48 and 72 hours after instillation and then daily until reversibility of the ocular reactions.

Results
The test item for the first rabbit was not severely irritating, so two additional animals were investigated. A very slight chemosis (day 1) and very slight redness of the conjunctiva (day 1 to day 3) were observed in 1/3 animals. Mean scores calculated for each animal over 24, 48 and 72 hours were 0.7/0/0 for redness of the conjunctiva and 0/0/0 for chemosis, iris lesions and corneal opacity.

Conclusion
Under the conditions of the experiment, HC Yellow n° 10 at 1% in 0.5 % methylcellulose was non-irritant to rabbit eyes.

Ref.: 3

3.3.3. Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429
Species/strain:	mouse – CBA/J
Group size:	5 females (10-11 weeks) per dose group
Test substance:	HC Yellow N°10
Batch:	N° 0508386
Purity:	99.8 %
Doses:	0.5%, 1.0%, 2.5%, 5.0%, 10% (w/v) in DMF
Positive control	alpha-hexylcinnamaldehyde (25% in DMF)
Vehicle control	DMF
GLP:	in compliance

25 µl of test material or vehicle control and positive control was applied to the dorsal surface of both ear lobes once daily for 3 consecutive days. After 2 days of resting, animals were injected with 250 µl ^{3}H-methyl thymidine in the tail vein. Mice were sacrificed 5 hours later. Draining lymph nodes were excised and pooled to prepare a single cell suspension for each group. Thymidine incorporation was measured by β-scintillation counting. The

disintegrations per minute per lymph node (DPM/node) was measured and expressed as the ratio of the control group (stimulation index, S.I. = dpm of treated group / dpm of control group).

Results

Treatment	Concentration (%)	Signs of local irritation	Stimulation Index (SI)
Test item	0.5	no	1.45
Test item	1	no	1.54
Test item	2.5	no	1.38
Test item	5	no	1.59
Test item	10	no	1.11
HCA	25	-	10.20

HC Yellow N°10 was not irritant in the preliminary test so the highest concentration retained for the main test was the maximal practicable concentration. No mortality and no clinical signs were observed during the study. No cutaneous reactions and no noteworthy increase in ear thickness were observed in the animals of the treated groups. No noteworthy lymphoproliferation and no dose-response relationship were noted at the tested concentrations.

Conclusion
Results of the LLNA test indicate that, under the conditions of the experiment, HC Yellow n° 10 is not a skin sensitizer.

Ref.: 4

3.3.4. Dermal / percutaneous absorption

***In vitro* Percutaneous Absorption**

Guideline:	OECD 428
Tissue:	human skin, 4 female donors, dermatomed skin thickness set at 400 µm
Tissue integrity:	tritiated water diffusion measurement
Method:	flow-through diffusion cells, exposed membrane area 0.64 cm^2
Receptor phase:	phosphate buffer saline (pH not specified) with 0.01 % sodium azide, sink conditions verified.
Test substance:	HC Yellow N°10 [^{14}C]- HC Yellow N°10
Batch:	non-radiolabelled: 0508386 [^{14}C] radiolabelled: CFQ14027 Batch 1
Purity:	non-radiolabelled: 99.8% [^{14}C] radiolabelled: 99.1%
Formulation:	semi-permanent (direct) hair dye under « in use » conditions
Concentration:	target 0.10% (experimental concentration 0.11%)
Dose applied:	20 mg / cm^2
Contact:	30 minutes, then washing of the skin surface, and monitoring of the diffusion during 23.5 hours.
No. of replicates:	8 cells (4 donors, 2 cells from each different donor)
Assay:	liquid scintillation

GLP: in compliance

After 30 minutes of exposure, the hair dye remaining on the skin surface was removed by washing. Twenty-four (24) hours after application, skin samples were removed and analysed by liquid scintillation counting to assess the cutaneous distribution of HC Yellow N°10. The mean total dermal absorption (sum of the amounts measured in the living epidermis, dermis and receptor fluid) of HC Yellow N°10 under the conditions of the experiment represented 0.061 ± 0.036 µgeq/cm² (0.28 ± 0.16% of the applied dose).

	Percentage of dose								
Replicate n°	A-1	A-2	A-3	A-4	B-1	B-2	B-3	B-4	
Donor n°	1	2	3	4	2	3	4	1	**Mean SD**
Skin wash	95.3	91.2	94.1	94.8	100.4	92.3	90.1	91.8	93.8 3.2
Cotton swabs	0.079	0.045	0.613	0.040	0.044	0.037	0.048	0.078	0.123 0.199
Donor compartment	0.010	0.034	0.178	0.005	0.003	0.005	0.003	0.010	0.031 0.060
Dislodgeable dose [1]	95.4	91.2	94.9	94.8	100.5	92.4	90.2	91.9	93.9 3.3
Tape strips	0.651	0.418	0.262	0.193	0.431	0.421	0.455	0.809	0.455 0.197
Unabsorbed dose [2]	96.1	91.7	95.2	95.0	100.9	92.8	90.7	92.7	94.4 3.2
Skin	0.206	0.261	0.109	0.188	0.206	0.102	0.179	0.232	0.185 0.055
Receptor fluid + Receptor wash	0.183	0.079	0.024	0.005	0.046	0.020	0.005	0.373	0.092 0.128
Total absorption [3]	0.389	0.341	0.133	0.193	0.252	0.122	0.184	0.605	0.277 0.163
Total recovery	96.5	92.0	95.3	95.2	101.2	92.9	90.8	93.3	94.7 3.2

[1] Amount in skin wash, cotton swabs and donor compartment wash
[2] Amount in dislodgeable dose and tape strips
[3] Amount in receptor fluid, receptor compartment wash and the skin (excluding tape strips)

	μg_{eq} / cm²								
Replicate n°	A-1	A-2	A-3	A-4	B-1	B-2	B-3	B-4	
Donor n°	1	2	3	4	2	3	4	1	**Mean SD**
Skin wash	21.0	20.1	20.8	20.9	22.2	20.5	20.0	20.3	20.7 0.7
Cotton swabs	0.017	0.010	0.135	0.009	0.010	0.008	0.011	0.017	0.027 0.044
Donor compartment	0.002	0.008	0.039	0.001	0.001	0.001	0.001	0.002	0.007 0.013
Dislodgeable dose [1]	21.1	20.1	20.9	20.9	22.3	20.5	20.0	20.4	20.8 0.7
Tape strips	0.144	0.092	0.058	0.043	0.096	0.093	0.101	0.179	0.101 0.044
Unabsorbed dose [2]	21.2	20.2	21.0	21.0	22.3	20.6	20.1	20.5	20.9 0.7
Skin	0.045	0.058	0.024	0.041	0.046	0.023	0.040	0.051	0.041 0.012
Receptor fluid + Receptor wash	0.040	0.018	0.005	0.001	0.010	0.004	0.001	0.083	0.020 0.028
Total absorption [3]	0.086	0.075	0.029	0.043	0.056	0.027	0.041	0.134	0.061 0.036
Total recovery	21.3	20.3	21.0	21.0	22.4	20.6	20.1	20.7	20.9 0.7

[1] Amount in skin wash, cotton swabs and donor compartment wash
[2] Amount in dislodgeable dose and tape strips
[3] Amount in receptor fluid, receptor compartment wash and the skin (excluding tape strips)

Conclusion
Under the described test conditions, a mean skin penetration of 0.061 ± 0.036 µgeq/cm² (0.28 ± 0.16% of the applied dose) was obtained for HC Yellow N°10, formulated as a semi-permanent (direct) hair dye. By summing up the amounts for receptor fluid, dermis and epidermis, the maximum skin absorption observed (A_{max}) was 0.134 µg/cm^2.

Ref.: 10

Comment
Although it was not performed according to the SCCP Notes of Guidance (only 8 skin samples were used), the study was considered acceptable. The maximum absorption

observed in the experiment (A_{max}) of 0.134 µg/cm^2 may be used for calculating the Margin of Safety.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

No data submitted

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

Guideline:	OECD 408
Species/strain:	Rat, Sprague-Dawley Crl CD (VAF plus)
Group size:	10/sex/group
Test substance:	Imexine FAH
Batch:	op T 10
Purity:	not specified in study report, > 99%
Dose:	0, 25, 100, 500 mg/kg
Vehicle:	0.5% methylcellulose
Route:	Gavage, 10 ml/kg/day.
Exposure:	92/93 days
GLP:	in compliance

These dose levels were selected on the basis of the results of 4-week preliminary study performed at 125, 500 and 2000 mg/kg/day, where significant changes in bodyweight and/or blood chemistry parameters at 2000 and/or 500 mg/kg/day were observed, and where the NOAEL was 125 mg/kg/day [17].
In the 13 week study, the dose was prepared daily. Animals were observed once daily for clinical signs and twice daily for mortality/morbidity. Body weight and food consumption were recorded weekly. Ophthalmologic evaluations on control and high-dose animals were performed before the treatment period and during week 13. Haematology, clinical chemistry and urinalysis evaluations were performed once during week 13.
At the end of the treatment period, all animals were killed and grossly examined. Selected organs were weighed. All animals were submitted to a complete macroscopic examination. All macroscopic lesions and required tissues from animals in the control and high-dose groups were evaluated microscopically; only macroscopic lesions and lungs, liver and kidneys were evaluated in the low and intermediate dose groups.

Results
No mortality occurred during the study. Dose-related (yellow to orange) changes in urine colour were observed in all groups. Yellow staining of tray liners, body and fur, ptyalism and black staining of faeces were seen dosed at 100 and 500 mg/kg/day. Bodyweight and food consumption were slightly higher than in the controls. Ophthalmological, haematology or blood chemistry parameters were comparable with control values. The yellow/orange colouration of urine prevented urinalysis for parameters determined using a clinistix®, but no abnormalities were found for those parameters that could be measured. Absolute and relative liver weights were significantly increased for both sexes at 500 mg/kg/day (+18% to +27% bw-related). In males, absolute spleen weights were increased at 100 and 500 mg/kg/day. Relative spleen weight increase was only seen at 500 mg/kg/day (+19%), but was not significant. There were no histopathological findings to correspond with these macroscopic changes.

Conclusion
The NOAEL for HC Yellow 10 in this study was considered to be 100 mg/kg/day.

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*

Bacterial gene mutation assay

Guideline:	OECD 471
Species/strain:	*Salmonella typhimurium* TA98, TA100, TA102, TA1535, and TA1537
Replicates:	triplicates in 2 individual experiments both in the presence and absence of metabolic activation.
Test substance:	HC Yellow No. 10 (1,5-di-(β-hydroxyethylamino)-2-nitro-4-chloro-benzene
Solvent:	DMSO
Batch:	0508386
Purity:	99.7%
Concentrations:	Experiment 1: 1.6 - 5000 µg/plate without and with S9-mix Experiment 2: 20.48 - 5000 µg/plate without and with S9-mix
Treatment:	Experiment 1: direct plate incorporation with 48 - 72 h incubation without and with S9-mix Experiment 2: direct plate incorporation with 48 - 72 h incubation without S9-mix pre-incubation method with 60 minutes pre-incubation and 48 - 72 h incubation with S9-mix.
GLP:	In compliance

HC Yellow No. 10 was investigated for the induction of gene mutations in *Salmonella typhimurium* (Ames test). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Test concentrations were based on the level of toxicity in a preliminary toxicity test with strain TA100 both without and with S9-mix. Toxicity was evaluated up to the prescribed maximum concentration of 5000 µg/plate on the basis of a thinning of the bacterial background lawn and a reduction in the number of revertant colonies. The data obtained with TA100 in this range-finder experiment were incorporated in experiment 1. Experiment 1 and experiment 2 without metabolic activation was performed with the direct plate incorporation method, experiment 2 with metabolic activation with the pre-incubation method. Negative and positive controls were in accordance with the guideline.

Results
Toxic effects in the form of a thinning of the background bacterial lawn were found in TA1537, TA100 (experiment 1) and TA102 (in experiment 2) in the presence of metabolic activation at the highest dose tested (5000 µg/plate). Precipitation of HC Yellow No. 10 was observed in all strains at the highest dose in the absence and presence of S9-mix, with the exception of TA98 in the presence of S9-mix in experiment 1.
In both experiments, no biological relevant, dose related and reproducible increases in revertants were observed in any of the strains tested in the absence or presence of metabolic activation.

Conclusion
Under the experimental conditions used HC Yellow No. 10 was not mutagenic in this gene mutation tests in bacteria.

Ref.: 6

In vitro* Mouse Lymphoma gene mutation assay (*tk* locus*)

Guideline:	OECD 476
Cells:	L5178Y $tk^{+/-}$ Mouse lymphoma cells
Replicates:	duplicates in 2 independent experiments
Test substance:	HC Yellow n° 10
Solvent:	DMSO
Batch:	0508386
Purity:	99.8%
Concentrations:	Experiment 1: 50 - 400 µg/ml (without S9-mix) 50 - 300 µg/ml (with S9-mix) Experiment 2: 50 - 400 µg/ml (without S9-mix) 50 - 400 µg/ml (with S9-mix)
Treatment:	3 h both without and with S9-mix; expression period 2 days and a selection period of 11 days.
GLP:	In compliance

HC Yellow n° 10 was assayed for mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of metabolic activation. Test concentrations were based on the results of a cytotoxicity range-finding experiment measuring relative total growth. In the main test, cells were treated for 3 h followed by an expression period of 2 days to fix the DNA damage into a stable *tk* mutation. Liver S9 fraction from Arachlor 1254-induced rats was used as exogenous metabolic activation system. Toxicity was measured as percentage relative total growth of the treated cultures relative to the total growth of the solvent control cultures. Negative and positive controls were in accordance with the OECD guideline.

Results
In the cytotoxicity range-finder experiment no significant changes in pH compared to concurrent controls were observed at the highest dose tested. In each main test the highest doses demonstrated precipitation of HC Yellow n° 10; for each separate test the highest dose evaluated for mutant colonies was the lowest dose with precipitation. Consequently, both in the absence and presence of S9-mix the appropriate level of toxicity (10-20% survival after the highest dose) was not reached.
No biological relevant increases in small or large mutant colonies were observed following treatment with HC Yellow n° 10 at any dose level tested, in the absence of S9-mix in both experiments and in the presence of S9-mix in experiment 1. In experiment 2, a small increase in mutant frequency was observed at 350 µg/ml in the presence of S9-mix. It was, however, considered not biologically relevant since it was not reproducible, no increase was found at the highest dose tested and there was no evidence of a linear trend over the entire dose range.

Conclusion
Under the experimental conditions used, HC Yellow n° 10 was considered not mutagenic in the mouse lymphoma assay at the *tk* locus.

Ref.: 7

Taken from opinion n° XXIV/1293/97, 20 May 1998

Chromosome aberration assay

The ability of induction of chromosome breakage in cultured Chinese Hamster Ovary (CHO) cells was tested in two independent tests with and without metabolic activation (S9 liver microsomes from beta-naphthoflavone and sodium phenobarbitone induced rats).

The dose levels tested were 20-200 µg/ml (without S9) and 150-1500 µg/ml (with S9). In the presence of S9-mix time contact was 3 hours. Cells were harvested after 24 or 48 hours after the start of dosing. The negative control was DMSO and the positive were mitomycine C (0.3 µg/ml) for cultures with metabolic activation and cyclophosphamide (15 µg/ml), respectively, for those without metabolic activation.
For analysis one hundred metaphases were scored from culture where possible. From each positive control only 25 metaphases were counted which gave statistically significant increases in aberrations compared to the negative controls.

Results
No statistically significant increases in aberrations were detected in the two experiments. As a conclusion the substance was evaluated as not showing clastogenic activity under these experimental conditions.

Ref. 5 (submission I)

In vitro micronucleus test

Guideline:	OECD 487 (draft 2004)
Cells:	human lymphocytes from 2 healthy, non-smoking female volunteers
Replicates:	duplicates in 2 independent experiments
Test substance:	HC Yellow n° 10
Solvent:	DMSO
Batch:	0508386
Purity:	99.7%
Concentrations:	Experiment 1: 0, 250, 350 and 400 µg/ml (without S9-mix) 0, 600, 650 and 750 µg/ml (with S9-mix) Experiment 2: 0, 400, 500, 600 and 700 µg/ml (without S9-mix) 0, 650, 750, 800 and 850 µg/ml (with S9-mix)
Treatment	Experiment 1: 24 h PHA followed by 20 + 28 h treatment (without S9-mix) 24 h PHA followed by 3 + 45 h treatment (with S9-mix) Experiment 2: 48 h PHA followed by 20 + 28 h treatment (without S9-mix) 48 h PHA followed by 3 + 45 h treatment (with S9-mix)
GLP:	In compliance

HC Yellow n° 10 has been investigated in the absence and presence of metabolic activation for the induction of micronuclei in cultured human lymphocytes. The suitable top concentrations for experiments 1 and 2 were based on the results of a cytotoxicity range-finding experiment measuring replication index (RI). To determine the test concentrations for micronucleus analysis in each separate experiment the RI is measured in cultures treated with increasing concentrations of HC Yellow n° 10. The top dose for micronucleus analysis was to be the one at which at least approximately 60% reduction in RI occurred or the highest dose tested. Two lower doses were selected so that a range of cytotoxicity from maximum (60%) to little or none is covered. Treatment periods were 20 h without and 3 h with S9-mix. Harvest times were 72 hours (experiment 1) or 96 hours (experiments 2) after the beginning of culture. The final 28 h of incubation were in the presence of cytochalasin B (at a final concentration of 6 µg/ml). Liver S9 fraction from Aroclor 1254-induced rats was used as exogenous metabolic activation system. Negative and positive controls were in accordance with the draft guideline.

Results
Measurements on post-treatment media in the absence or presence of S9-mix indicated that HC Yellow n° 10 had no effect on osmolarity or pH as compared to concurrent vehicle controls.

In both experiments, biologically relevant increases in the number of micronucleated binucleate cells compared to concurrent control values were not found at any concentration tested both in the absence or presence of S9-mix. In the absence of metabolic activation in experiment 1 at 350 µg/mL and experiment 2 at 400 µg/ml a small but statistically significant increase in the number of binuclear cells with micronuclei compared to the concurrent control was observed. As the numbers of micronucleated binuclear cells were within the historical control range and were not observed to be dose related, they were considered not biologically relevant.

Conclusion
Under the experimental conditions used HC Yellow n° 10 did not induce micronuclei and, consequently, is not genotoxic (clastogenic and/or aneugenic) in cultured human peripheral lymphocytes *in vitro*.

Ref.: 8

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

No data submitted

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

Guideline:	OECD 414 (2001)
Species/strain:	Rat, Sprague-Dawley Ico SD (IOPS Caw)
Group size:	25 mated female per dose
Test substance:	HC Yellow n° 10
Batch:	0508386
Purity:	99.8%
Dose:	0, 100, 300 or 1000 mg/kg/day
Vehicle:	0.5% aqueous carboxymethylcellulose
Route:	gavage, 5 ml/kg/day
Exposure:	Gestation day (GD) 6-19
GLP:	in compliance

Solutions were prepared at least weekly, stored in the dark at 4 °C. The dose levels were selected on the basis of the results of a previous inadequate study in which rats were given HC Yellow n° 10 at 125, 500 or 2000 mg/kg/day [21, (1998 opinion, ref 7)]. In that study, findings at 2000 mg/kg/day were indicative of slight maternal toxicity during the dosing period (decreased body weight gain (-26% from controls) and feed intake (-15%)) and of slight foetal developmental toxicity.
Animals were observed twice daily for mortality/morbidity. Clinical signs were checked once daily. Food consumption and body weight were recorded at designated intervals during gestation.
On day 20 of gestation, the animals were killed and examined macroscopically. Foetuses were removed by Caesarean section. The following litter parameters were recorded: number of corpora lutea, number of implantation sites, number and distribution of early and late resorptions, and number of dead and live foetuses. Foetuses were weighed, sexed and

submitted to external, soft tissue and skeletal examinations. Statistical analysis was performed on the following data: body weight, food consumption, litter data, and the proportions of live foetuses, pre- and post-implantation loss and foetuses with abnormalities. The homogeneity and concentrations of test substance preparations were verified.

Results
The chemical analysis of the dose formulations administered showed achieved concentrations close to the intended nominal values (deviations within -0.4% to 4.6%).
There were no deaths during the study. Clinical observations included a yellow staining of the fur at all dose levels. There were no other noteworthy clinical signs, other than yellow coloured faeces and urine noted on occasions in the three treated groups.
Rats given 1000 mg/kg/day showed a transient and significant reduction in maternal body weight gain (5/23 pregnant rats lost weight over the first three days of the treatment period) and a lower mean body weight gain (-6%) compared to controls over the dosing period. Food consumption was reduced in the 1000 mg/kg/day group throughout the treatment period, particularly during the first three days (mean food intake in this group: -13.4% compared with controls at the end of the study). Body weight gain and food intake were not significantly affected in the lower dose groups. No abnormal *post mortem* findings were noted.
The pregnancy rate (23/25, 23/25, 25/25, and 23/25 at 0, 100, 300, and 1000 mg/kg/day, respectively) was not affected by treatment. All females had live foetuses at term. There were no dead foetuses in any group. The overall incidence of early resorptions was marginally higher but not statistically significant in the groups given 300 and 1000 mg/kg/day than in controls (8.0 and 9.6% of implantations respectively compared with controls 5.5%). The litter distribution of the resorptions did not suggest treatment effects. There were two late resorptions in each of the 300 and 1000 mg/kg/day groups and none in the 100 mg/kg/day and control groups. The percentage post-implantation loss was greater in the 300 and 1000 mg/kg/day than in controls, reflecting the above-mentioned incidental differences in resorption incidences. The mean number of live foetuses per litter was nonetheless high (at least 12) in the three treated groups and remained comparable with the concurrent control value.
Mean foetal weight and the mean gravid uterus weight were comparable in all groups. The mean proportion of male foetuses was lower but not statistically significant in the 300 and 1000 mg/kg/day groups (44% and 45% compared with 50% in the control group).
There were two malformed foetuses in the 300 mg/kg/day group, one in each of the 100 and 1000 mg/kg/day groups and one in the control group. The incidences and types of malformation found did not suggest an involvement of the test item. One foetus from each of the intermediate and high dose groups had major thoracic defects, a low dose foetus had microphtalmia and the other malformed intermediate dose foetus had a cleft palate. Foetal ossification was delayed in the group given 1000 mg/kg/day, as indicated in the incidences of foetuses with generalised reduced ossification or localised incomplete ossification of the skull, vertebrae, sternum, paws and/or pelvis. Occasional foetuses from the 300 mg/kg/day group were similarly affected, but the differences with respect to the control group were minimal. The low dose group was unaffected. The incidences of skeletal or soft tissue foetal abnormalities did not show any treatment-related trends and were considered unlikely to cause permanent defects.
The No Observed Adverse Effect Level (NOAEL) for HC Yellow n° 10 in this study for maternal toxicity and embryo-foetal development was 100 mg/kg/day. Doses up to 1000 mg/kg/day to pregnant Sprague-Dawley rats were not teratogenic.

Ref.: 9

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

(HC Yellow n° 11)
(Direct / semi-permanent)

Maximum absorption through the skin	**A (µg/cm^2)**	**=**	**0.134 µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**700 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.094 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.0016 mg/kg**
No observed adverse effect level (90-day, oral, rat)	**NOAEL**	**=**	**100 mg/kg**
Margin of Safety	**NOAEL / SED**	**=**	**62500**

3.3.14. Discussion

Physico-chemical properties
HC Yellow n° 10 is used in semi-permanent (direct) hair colouring products at a general on-head concentration of 0.1%.
HC Yellow n° 10 is a secondary amine, and thus is prone to nitrosation. It should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb.
No data was provided on the stability of HC Yellow n° 10 in marketed products.

General toxicity
The maximum non-lethal dose of HC Yellow N°10 following single oral gavage to fasted rats was 2000 mg/kg.
The NOAEL for HC Yellow 10 in a 90-day study was considered to be 100 mg/kg/day.
The No Observed Adverse Effect Level (NOAEL) for HC Yellow 10 for maternal toxicity and embryo-foetal development was 100 mg/kg/day. Doses up to 1000 mg/kg/day to pregnant Sprague-Dawley rats were not teratogenic.

Irritation / sensitisation
Under the conditions of the respective experiments, HC Yellow n° 10 at 1% in 0.5% methylcellulose was non-irritant to the rabbit skin following a single dermal application and non-irritant to rabbit eyes.
Results of the LLNA test indicate that, under the conditions of the experiment, HC Yellow n° 10 is not a skin sensitizer.

Dermal absorption
The maximum absorption (A_{max}) observed in the *in vitro* percutaneous absorption experiment, 0.134 µg/cm², has been used for calculating the Margin of Safety.

Mutagenicity / genotoxicity
Overall, the genotoxicity of HC Yellow n° 10 is sufficiently investigated in valid genotoxicity tests including the 3 types of mutations: gene mutations, chromosome aberrations and aneuploidy. HC Yellow n° 10 did not induce gene mutations in bacteria or mammalian cells nor micronuclei in an *in vitro* micronucleus test.
Consequently, HC Yellow n° 10 can be considered to have no genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No data submitted

4. CONCLUSION

The SCCP is of the opinion that the use of HC Yellow n°10 itself as a semi-permanent hair dye at an on-head concentration of maximum 0.1% does not pose a risk to the health of the consumer.

HC Yellow n° 10 is a secondary amine. It should not be used in combination with nitrosating substances. The nitrosamine content should be < 50 ppb.

5. MINORITY OPINION

Not applicable

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