SCCS/1322/10
Revision of 22 March 2011

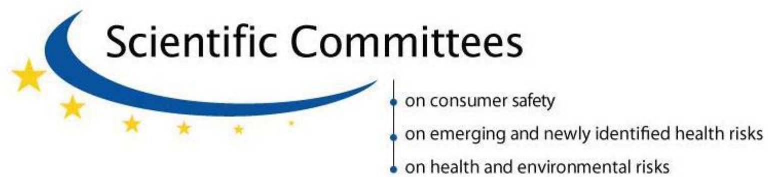
Scientific Committee on Consumer Safety

SCCS

OPINION on

HC Yellow n° 13

COLIPA n° B102



The SCCS adopted this opinion at 9th plenary meeting on 14 December 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 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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ISSN 1831-4767 ISBN 978-92-79-32654-7

Doi:10.2772/30935 ND-AQ-09-035-EN-N

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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

ACKNOWLEDGMENTS

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Keywords: SCCS, scientific opinion, hair dye, B102, HC Yellow n° 13, CAS 10442-83-8, EC 443-760-2, directive 76/768/EEC

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on HC Yellow n° 13, 14 December 2010

This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

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1. BACKGROUND

Submission I for HC Yellow n° 13 with the chemical name 4-(2'-Hydroxyethyl)-amino-3-nitrotrifluormethylbenzene was submitted in October 1993 by COLIPA [1, 2].

Submission II for HC Yellow n° 13 was submitted in April 2002 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 an opinion (SCCNFP/0689/03, final) with the conclusion that:

"HC Yellow n° 13 consists for more than 99 % of N-(2-hydroxyethyl)-2-nitro-4-trifluormethylaniline. However, relevant physico-chemical parameters are not given. Purity of the test material in several studies is not reported. The dye is a secondary alkanolamine, and thus, it is prone to nitrosation. No data is provided on the nitrosamine content of the dye and in hair dye formulations. The data provided on stability are insufficient.
HC Yellow n° 13 was found to be of low acute oral and dermal toxicity in rats. No signs of eye or skin irritation were observed. The sensitisation test results were negative. In a 90-day oral toxicity study in rats, 30 mg/kg bw was the NOAEL. In a rat teratogenicity study, no structural abnormalities were observed in the foetuses, however, there were slight indications of developmental retardations following administration of 30 and 90 mg/kg/bw/day during the critical days of organogenesis. 10 mg/kg bw was the level without developmental anomalies.
Skin penetration (rat in vivo study, using 14C-labelled a.i.) indicated a maximum penetration of 2.5 µg/cm2 for oxidative hair dyes (max. in-use concentration 2.5%), and of 9.69 µg/cm2 for colour setting lotions (max. in-use concentration of 5%).
HC Yellow n° 13 was tested in procaryotic cells for gene mutation in several tester strains of S. typhimurium. The test is unsuitable for genotoxicity evaluation. A second Bacterial Reverse Mutation Test was provided and was acceptable for evaluation. Based on the reversion rate, it is concluded that HC Yellow n° 13 dissolved in DMSO is negative in any S. typhimurium tester strains in the absence or the presence of S9 mix. The earlier in vitro mammalian chromosomal aberration test is negative. However, the test is unsuitable for genotoxicity evaluation. A more recent suitable in vitro mammalian chromosomal aberration test has been provided in submission II with HC Yellow n° 13 in DMSO. It is positive for clastogenicity. HC Yellow n° 13 gave negative results in the mammalian erythrocyte micronucleus test. However, the study did not demonstrate that bone marrow was reached by the test agent."

Submission III for HC Yellow n° 13 was submitted by COLIPA in July 2005. According to this submission the substance is used as:

a) *a non-reactive hair colouring agent ("direct dye") in non-oxidative hair dye formulations at a maximum on-head concentration of 2.5%. It is common practice to apply 35 to 50 g of the product over a period of 30 minutes followed by rinse off with water and shampoo. The application may be repeated at weekly intervals.*
b) *a non-reactive hair colouring agent ("direct dye") in oxidative hair dye formulations at a maximum on-head concentration of 2.5%. The colouring component and a developer (hydrogen peroxide) are mixed in ratios between 1:1 to 1:3. It is common practice to apply up to 100 g of the finished mixed product for a period of 30 to 45 minutes followed by rinse off with water and shampoo. The application may be repeated at monthly intervals.*

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

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° 13 safe for use as a non-oxidative hair dye with an on-head concentration of maximum 2.5% taken into account the scientific data provided?*

2. *Does the SCCS consider HC Yellow n° 13 safe for use in oxidative hair dye products with an on-head concentration of maximum 2.5% taken into account the scientific data provided?*

3. *Does the SCCS recommend any further restrictions with regard to the use of HC Yellow n° 13 in any non-oxidative or oxidative hair dye formulations?*

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° 13 (INCI name)

3.1.1.2. Chemical names

N-(2-Hydroxyethyl)-2-nitro-4-trifluormethyl-aniline
1-(2-Hydroxyethyl)amino-2-nitro-4-trifluormethylbenzene

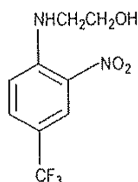
3.1.1.3 Trade names and abbreviations

Fluorgelb II
Cos 128
COLIPA B102

3.1.1.4 CAS /EC number

CAS: 10442-83-8
EC: 443-760-2

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

Formula: $C_9H_9F_3N_2O_3$

3.1.2 Physical form

Yellow crystalline powder

3.1.3 Molecular weight

Molecular weight: 250.18 g/mol

3.1.4 Purity, composition and substance codes

4 batches were tested for the purity and impurities. The chemical characterisation was performed by NMR and IR. UV-spectra of the 4 batches were similar.

	G2001/011-001: batch 97/92/1094	G2001/011-002: batch AR902,	02001/011-003: batch AZ212,	G2001/011-004: batch AR822, Eingang: 05.09.97
NMR content (% w/w)	99.7	99.8	99.6	99.9
HPLC purity (% peak area)				
210 nm	99.5	99.8	99.8	99.8
254 nm	99.9	100	100	100
418 nm	99.8	100	100	100
Loss on drying (% w/w)	0.12	0.06	0.06	n.a
Water content (% w/w)	0.06	0.02	0.02	n.a
Sulfated ash (% w/w)	0.005	0.004	0.003	n.a.

n.a.: not analysed

Chromatograms of the samples at the HPLC purity test showed at the 210, 254, and 418 nm detection wavelength one main peak with 99.5 - 100 area % and minor peaks with 0.01 - 0.34 area % which might represent traces of impurities.

Declaration by the Applicant

Deduced specification
Purity
HPLC quantitative: > 98 % w/w
HPLC qualitative (254 nm): > 99 %
Solvent content: < 1 %

3.1.5 Impurities / accompanying contaminants

1-Chloro-2-nitro-4-trifluoro-methylbenzene < 100 ppm

3.1.6 Solubility

Water solubility: 506 mg/L (20°C) determined by EC –A.6 method
DMSO solubility: 50 mg/ml

See Ref 3

3.1.7 Partition coefficient (Log P_{ow})

Log $P_{o/w}$: 2.54 (pH 6.5 -7.1, 23°C) determined by EC –A.8 method

3.1.8 Additional physicochemical specifications

Melting point: 74.7 °C
Boiling point: 227.1 °C
Flammability: not highly flammable
Flash point: > 400 °C
Vapour pressure: 3.1×10^{-8} hPa (20 °C)
Relative density: 1.45 (20 °C)
Surface tension (water): 42.4 nM/m (20 °C)
Viscosity: /
Oxidising properties: not oxidising
pKa: /
Refractive index: /
UV/ Vis spectrum: absorption maxima at 240nm and 408 nm

3.1.9. Stability

HC Yellow n° 13 suspensions in 0.5% aqueous CMC used for the 90 day oral toxicity testing were shown to be homogeneous and stable (variation ± 10%) during the study.

The stability of a solution of approximately 50 mg/L HC Yellow n° 13 in DMSO was examined at room temperature. After a storage period of 4 h, the recovery of the dye was 100.6%.
The stability of HC Yellow n° 13 was tested in a hair dye formulation in the presence of hydrogen peroxide for 15, 30 and 45 minutes. The test results demonstrate the stability of HC Yellow n° 13 during a 45 minute test period.

General Comments on Physico-chemical characterisation

- HC Yellow n° 13 is a secondary amine, and thus, it is prone to nitrosation. Nitrosamine content in HC Yellow n° 13 has not been reported.
- Stability of HC Yellow n° 13 in typical hair dye formulations has not been demonstrated.

3.2. Function and uses

HC Yellow n° 13 is used as a hair colouring agent ("Direct Dye") in non-oxidative and in oxidative hair dye formulations at a maximum on-head concentration of 2.5%.

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCNFP/0689/03

Guideline: OECD 401 (1987)
Species: Wistar rats, Crl: (WI)BR
Group size: 5 males + 5 females
Material: Fluorgelb II in 2% gum Arabic
Batch: AR 902
Purity: 99.7%
Dose: 2000 mg/kg bw in a volume of 10 ml/kg
Observ. period: 14 days
GLP: in compliance
Study date: 1990

Groups of 5 male and 5 female rats received a single oral dose of 2000 mg/kg bw. The animals were observed daily for 14 days for clinical abnormalities and mortality. Body weights and macroscopic observations were recorded.

Results
No mortalities were observed. The treatment caused lethargy, piloerection, abnormal posture and reduced righting reflex for up to 6 hours after dosing. The LD50 was greater than 2000 mg/kg bw.

Ref.: 16

3.3.1.2. Acute dermal toxicity

Taken from SCCNFP/0689/03

Guideline:	OECD 402 (1987)
Species:	Sprague Dawley rats, Him:OFA
Group size:	5 males + 5 females
Material:	Fluorgelb II moistened with distilled water
Batch:	AR 902
Purity:	99.7%
Dose:	2000 mg/kg bw on an area of 5 x 6 cm
Observ. period:	14 days
GLP:	in compliance
Study date:	1992

Moistened Fluorgelb II was administered, at a dose of 2000 mg/kg bw, under an occlusive patch to a shaven area on the back of 5 male and 5 female Sprague Dawley rats for 24 hours. The animals were observed twice daily for 14 days for clinical abnormalities and mortality. Body weights and macroscopic observations were recorded.

Results
All animals survived until the end of the study. Chromodacryorrhea was noted in 3 males and 2 females. In 3 females body weight gain was lower than for controls. Fur and tails were stained in all animals. The LD50 was reported to be greater than 2000 mg/kg bw.

Ref.: 17

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Taken from SCCNFP/0689/03

Guideline:	OECD n° 404
Species:	New Zealand white rabbit
Group size:	6 (sex not specified)
Material:	5% Fluorgelb II suspended in propylene glycol (pH 6.5)
Batch:	AR 902
Purity:	99.7 %
Dose:	0.5 ml on 6 cm^2
GLP:	in compliance
Study date:	1990

0.5 ml of a 5% solution of the test material in propylene glycol was topically applied to the clipped back of 6 rabbits under occlusive conditions for 4 hours. The material residues were then washed off. Skin reactions were recorded 30 - 60 min and 24, 48 and 72 h after removal of the rubber sheet.

Results
No signs of erythema or oedema were observed. A 5% solution of the test material was not irritant to rabbit skin.

Ref.: 18

3.3.2.2. Mucous membrane irritation

Taken from SCCNFP/0689/03

Test on diluted material

Guideline: OECD n° 405
Species: New Zealand white rabbit
Route: eye
Group size: 6 (sex not specified)
Material: 5% Fluorgelb II suspended in propylene glycol (pH 6.8)
Batch: AR 902
Purity: 99.7%
Dose: 0.1 ml
GLP: in compliance
Study date: 1990

0.1 ml of a 5% dilution of the test material in propylene glycol was instilled into the left eye of 6 New Zealand White rabbits. In 3 animals it was washed out after 4 seconds. The untreated right eye served as control. Eye reactions were recorded at 1, 24, 48 and 72 hours after treatment.

Results
Slight redness and chemosis of the conjuctivae was observed. According to 83/467/EEC, 5% Fluorgelb II in propyleneglycol was classified as not irritant.

Ref.: 19

Test on undiluted material

Guideline: OECD n° 405
Species: rabbit (New Zealand white)
Route: eye
Group size: 3 females
Material: Fluorgelb II
Batch: AR 902
Purity: 99.7%
Dose: Approximately 0.1 ml
GLP: in compliance
Study date: 1991

The equivalent of 0.1 ml (actual amounts 52, 64 and 80 mg) of the undiluted test material was instilled into the right eye of 3 rabbits. The untreated left eye served as control. Eye reactions were read at 1, 24, 48 and 72 hours after treatment.

Results
Mild reactions were seen with slight effects on the cornea, iris, and conjunctiva reported. In all cases the mean scores were below the thresholds defined in Directive 83/467/EEC for classification as "irritant".

Ref.: 20

3.3.3. Skin sensitisation

Taken from SCCNFP/0689/03

Magnusson and Kligman Maximisation test

Guideline:	OECD n° 406	
Species:	guinea pigs (Pirbright BOR:DHPW)	
Group size:	10 males and 10 females	
Material:	Fluorgelb II	
Batch:	AR 902	
Purity:	99.7%	
Concentrations:	Intradermal induction:	10% test material in propylene glycol
	Topical induction:	0.5 g undiluted test material
	Challenge:	0.2 g undiluted test material and 0.2 ml 5% solution in propylene glycol
GLP:	in compliance	
Study date:	89	

The systemic induction phase consisted of 3 pairs of 2 intradermal injections (0.1 ml each) on the clipped dorsal shoulder region of the animals. The injections contained: 1) the test material (10%) in propylene glycol; 2) the test material (10%) in Freund's complete adjuvant, 1:2 diluted; 3) Freund's complete adjuvant in distilled water (1:1).

7 days later the pure test material was topically applied and occluded for 48 hours. The controls received similar treatments but received vehicle (without test substance) only. The challenge was carried out 3 weeks after the first intradermal treatment. 0.2 g of the pure test material and 0.2 ml of a 5% solution in propylene glycol were topically applied to the left flank for 24 hours under an occlusive patch. Reactions were recorded at 24 and 48 hours after the last application.

Results
Sporadic, slight erythema was observed in both test and control animals at comparable incidence.

Conclusion
The test material was considered non-sensitising.

Ref.: 21

Comment
This study was not fully in compliance with OECD guideline 406, which specifies that, when testing a non-irritating material, local irritation should be induced 24 hours before the topical induction.
The sensitisation data in the dossier was generated without inducing irritation during induction.

3.3.4. Dermal / percutaneous absorption

Guideline:	OECD 428 (2004)
Tissue:	Porcine (Schweizer Landedelschwein, female) back and flank skin (thickness: mean 1000 µm)
Group size:	6 (five for the formulation containing the test item and one for the blank formulation)
Skin integrity:	tritiated water
Diffusion cell:	Diffusion Teflon-chambers
Test substance:	Fluorgelb (WR18213) tested at a concentration of 2.5 %

Batch:	AZ212
Purity:	100 % (HPLC at 254 nm)
Radiochemical	/
Test item:	typical oxidative hair dye formulation
Dose volume:	100 mg/cm² of formulation
Receptor fluid:	physiological receptor fluid
Solubility receptor fluid:	0.769 mg/ml
Stability receptor fluid:	stable
Method of Analysis:	HPLC
GLP:	In compliance
Study date:	May 2005-June2005

The integrity of each skin preparation was demonstrated with tritiated water resulting in 0.4 to 1.9% of the applied dose found in the receptor fluids, which was within the limit of acceptance (< 2.0%) for all 6 skin samples.
400 mg of formulation (= 100 mg/cm²) containing 2.5% Fluorgelb (WR18213) was applied once to the skin samples (4 cm²) in a typical cream formulation for oxidative hair dyes.
Six chambers were investigated. The receptor solution (physiological phosphate buffer containing NaCl and antibiotics) was pumped through the receptor chamber at a rate of 5 ml/h.
Sixty minutes after test 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. The washing 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 and analysed immediately. At termination of the experiment, the skin was heat-treated and the 'upper skin' (stratum corneum and upper stratum germinativum) was 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
All samples/tissue extracts were analysed by HPLC.
The total balance (total recovery) was 100.21 ± 3.41% of the dose applied.
The majority of the applied dose of Fluorgelb (WR18213) remained on the skin surface (100.10 ± 3.40% of the applied dose or 2.503 ± 0.085 mg/cm²). After 72 h, 1.65 ± 0.11 µg/cm² had penetrated into the receptor fluid, 0.04 ± 0.03 µg/cm² in the upper skin, and 0.92 ± 0.42 µg/cm² was recovered in the lower skin.
The amount 2.61 ± 0.56 µg/cm² (receptor fluid + upper skin + lower skin) of Fluorgelb (WR18213) was considered to be bioavailable.

Ref.: 22

Comment
The amount 2.61 ± 0.56 µg/cm² (receptor fluid + upper skin + lower skin) of Fluorgelb (WR18213) was considered to be bioavailable under oxidative conditions in a formulation containing 2.5% Fluorgelb. However, only 5 chambers were used and the dose of formulation was too high. Therefore, the mean+2SD (3.73 µg/cm²) should be used in calculating the MOS.

Guideline:	OECD 428 (2004)
Tissue:	Porcine back and flank skin (thickness: 1000 µm)
Group size:	6 (five for the formulation containing the test item and one for the blank formulation) per study
Skin integrity:	tritiated water
Diffusion cell:	Diffusion Teflon-chambers
Test substance:	Fluorgelb (WR18213) tested at a concentration of 2.5%

Batch: AZ212
Purity: 100% (HPLC at 254 nm)
Radiochemical /
Test item: typical non-oxidative hair dye formulation
Dose volume: 100 mg/cm² of formulation
Receptor fluid: physiological receptor fluid
Solubility receptor fluid: 0.769 mg/ml
Stability receptor fluid: stable
Method of Analysis: HPLC
GLP: In compliance
Study date: June 2005

The integrity of each skin preparation was demonstrated with tritiated water resulting in 0.6 to 0.9% of the applied dose found in the receptor fluids, which was within the limit of acceptance (< 2.0%) for all 6 skin samples.
400 mg of formulation (= 100 mg/cm²) containing 2.5% Fluorgelb (WR18213) was applied once to the skin samples (4 cm²) in a typical cream formulation for non-oxidative hair dyes. The experiment was carried out as in that under oxidative conditions.

Results
The total balance (total recovery) 94.37 ± 5.45% of the dose applied to the pig skin samples confirmed the validity of the experiment.
The majority of the applied dose of Fluorgelb (WR18213) remained on the skin surface (94.2 ± 5.47% of the applied dose or 2.357 ± 0.137 mg/cm². After 72 h, 1.87 ± 0.56 µg/cm² had penetrated into the receptor fluid, 0.01 ± 0.01 µg/cm² in the upper skin, and 0.07 ± 0.02 µg/cm² was recovered in the lower skin.
The amount 1.95 ± 0.59 µg/cm² (receptor fluid + upper skin + lower skin) of Fluorgelb (WR18213) was considered to be bioavailable.

Ref.: 23

Comment
The amount 1.95 ± 0.59 µg/cm² (receptor fluid + upper skin + lower skin) of Fluorgelb (WR18213) was considered to be bioavailable under non-oxidative conditions in a formulation containing 2.5% Fluorgelb. However, only 5 chambers were used and the dose of formulation was too high. Therefore, the mean + 2SD (3.13 µg/cm²) should be used in calculating the MOS.

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

Taken from SCCNFP/0689/03

Guideline: OECD n° 408
Species: Wistar rats, Crl: (WI) BR
Group sizes: 15 males and 15 females (+ 10 males and 10 females for recovery high dose and control group)
Material: Fluorgelb II in 0.5% aqueous sodium carboxymethylcellulose
Batch: AZ 212
Purity: > 99%
Dose levels: 0, 10, 30 and 90 mg/kg bw in a volume of 10 ml/kg
Exposure: 5 days per week for 90-92 days

GLP: in compliance
Study date: 1991

Fluorgelb II, in 0.5% aqueous sodium carboxymethylcellulose, was administered by oral gavage to groups of 15 male and 15 female rats at doses of 10, 30 and 90 mg/kg bw, 5 days per week for 90-92 days. The high dose group and the control group included an additional 10 males and 10 females for observation in a 4-week recovery period. Controls received the vehicle only. The following investigations were performed: daily observations, ophthalmoscopy, bodyweights, food consumption, haematology, clinical chemistry and urinalysis (at several stages), gross pathological examination, organ weight determination and histopathology.

Results
No animals died during the study. Urine samples of all treated animals were dark yellow throughout the administration period. Body weights and food consumption were unaffected by treatment. Blood, urine and clinical chemistry data were all within the normal range of variation with the exception of serum cholesterol in high dose males, which was elevated and did not return to normal following the 4-week recovery period.
No ophthalmological or macroscopic changes were observed. There was no effect on either the absolute or relative organ weights in treated rats compared with controls.
Islet cell degeneration, accompanied by inflammation or fibrosis of the endocrine pancreas was observed in two of the male rats treated with 90 mg/kg bw. These changes were accompanied by a high but not statistically significant blood glucose level and were considered to be material related. No pancreatic changes were found in any intermediate-dose animals. No other treatment related effects were reported. The dose of 30 mg/kg bw/day was considered as NOAEL

Ref.: 25

Comment
The NOAEL was adjusted to 21 mg/kg bw/day because of only 5 days treatment per week.

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 Assay

Guideline: OECD 471
Species/strain: *Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA 1538
Replicates: Triplicate plates, 2 independent tests
Test substance: N-(2-hydroxyethyl)-2-nitro-4-(trifluormethyl)aniline
Batch: 97/92/1094
Purity: 99.9% (GC/HPLC)
Vehicle: DMSO
Concentrations: experiment 1: 1, 10, 100, 1000 and 5000 µg/plate with and without S9-mix
experiment 2: 30, 100, 300, 1000 and 3000 µg/plate with and without S9-mix
Treatment: direct plate incorporation with 48 h incubation without and with S9-mix
GLP: in compliance
Study date: 2 November 1995 – 15 December 1995

N-(2-hydroxyethyl)-2-nitro-4-(trifluormethyl)aniline has been investigated for gene mutations in *S. typhimurium,* using the direct plate incorporation method both with or without S9-mix. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. Both experiments were performed with the direct plate incorporation method. Toxicity was evaluated on the basis of a reduction in the number of revertant colonies and a qualitative evaluation of the bacterial lawn. Negative and positive controls are in accordance with the OECD guideline.

Results
Toxicity as evidenced by a reduction in the number of spontaneous revertants per plate and an abnormal background lawn was observed for every tester strains at dose levels of 5000 (experiment 1) and 3000 µg/plate (experiment 2) both without and with S9-mix. Without and with S9-mix N-(2-hydroxyethyl)-2-nitro-4-(trifluormethyl)aniline did not induce a dose related or biologically relevant increase in revertant numbers at any dose in any of the *S. typhimurium* tester strains in both experiments performed.

Conclusions
Under the experimental conditions used N-(2-hydroxyethyl)-2-nitro-4-(trifluormethyl)aniline was not mutagenic in this gene mutation tests in bacteria.

Ref.: 26

***In vitro* Mammalian Chromosome Aberration Test**

Guideline:	OECD 473 (1997).
Species/strain:	Chinese Hamster V79 Cells
Replicates:	Duplicate cultures
Test substance:	Fluorgelb II, 89105
Batch:	CH 97/92/1094
Purity:	99.7%
Vehicle:	DMSO
Concentrations:	50, 100 and 150 µg/ml with and without metabolic activation.
Treatment:	4 h both in absence and presence of S9-mix; harvest time 18 h after start of treatment.
GLP:	in compliance
Study date:	21 August 2001 – 17 December 2001

Fluorgelb II has been investigated for induction of chromosomal aberrations in V79 cells. Liver S9 fraction from rats induced with Phenobarbital/β-naphthoflavone was used as the exogenous metabolic activation system. Fluorgelb II test concentrations were based on the results of a pre-test with concentrations between 20.3 and 2600 µg/ml (corresponding to circa 10 mM, the prescribed maximum concentration) in the presence and absence of S9-mix using reduced cell numbers as indication for toxicity. In the chromosome aberration test the treatment period was 4 h with and without S9-mix; cultures were harvested 18 h after the start of treatment. The final 2.5 h before harvest culture was in the presence of Colcemid (at a final concentration of 0.2 µg/ml) to block cells at metaphase of mitosis. Toxicity was determined by measuring the reduction in mitotic index (MI). Chromosome (metaphase) preparations were stained with Giemsa and examined microscopically for chromosomal aberrations. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-test no precipitation occurred; at the top concentration, no influence on the pH or osmolarity was noted. In the pre-test clear toxic effects were seen with 325 µg/ml and above; therefore, 250 µg/ml was chosen as highest concentration in the chromosome aberration test. Toxic effects as evidenced by a reduction in cell numbers were observed in the presence of S9-mix at 150 µg/ml. A reduction in the mitotic index relative to the

concurrent control was not noted in the absence of metabolic activation, in the presence of metabolic activation at the top dose a 73.5 % reduction of the mitotic index was observed.
In the presence, but not in the absence, of S9-mix a biologically relevant, statistically significant and dose dependent increase in the number of cells with chromosome aberrations was found. No biologically relevant increase in the number of polyploid metaphases was recorded.

Conclusion
Under the experimental conditions used, Fluorgelb II induced an increase in cells with chromosomal aberrations and, consequently, is genotoxic (clastogenic) in V79 cells *in vitro*.
Ref.: 27

Comment
The required 50% reduction in mitotic index as measure for sufficient exposure was not reached. Since Fluorgelb II was considered to be genotoxic after 4 h treatment a second experiment with continuous treatment was not performed.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

***In vivo* Mammalian Erythrocytes Micronucleus Test**

Guideline:	OECD 474
Species/strain:	NMRI mice
Group size:	6 mice/sex/group
Test substance:	Fluorgelb II
Batch:	97/92/1094
Purity:	> 99.5 %
Vehicle:	PEG 400
Dose level:	0, 375, 750 and 1500 mg/kg bw/day
Route:	oral, three times at 24 h intervals
Sampling:	24 h after the final treatment
GLP:	in compliance
Study date:	16 April 2002 - 5 November 2002

Fluorgelb II has been investigated for the induction of micronuclei in bone marrow cells of mice. Test concentrations were based on the acute toxicity in a pre-experiment for toxicity with 2 animals per sex/group. The animals were treated orally three times at 24 h intervals and were examined for acute toxic systems at intervals around 1, 2-4, 6 and 24 h after each administration of Fluorgelb II. In the main experiment mice were exposed orally three times at 24 h intervals to 0, 375, 750 and 1500 mg/kg bw/day. Bone marrow cells were collected 24 h after the last treatment. The animals of the highest dose group were examined for acute toxic symptoms 1, 2-4, 6 and 24 h after each administration. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and total erythrocytes (PCE/TE). Bone marrow preparations were stained with May-Grünwald/Giemsa and examined microscopically for the PCE/TE ratio and micronuclei. Five mice/sex/group were analysed; the remaining 6th animals of each group were only evaluated in case a mouse died spontaneously. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-experiment for toxicity the mice treated with 1500 mg/kg bw/day showed reduction of spontaneous activity and ruffled fur, abdominal position (after the 2nd application only), eyelid closure (after the 2nd and 3rd application only) and apathy (after the 2nd and 3rd application only). Based on these finding and since all mice survived, 1500 mg/kg bw/day was chosen as the top dose.
In the main experiment, one animal died after the first application of the highest dose 1500 mg/kg bw/day. The surviving mice treated with 1500 mg/kg bw/day showed reduction of

spontaneous activity, abdominal position, eyelid closure, ruffled fur and apathy (after the 2nd and 3rd application only). After the 2nd and 3rd application the mice had yellow coloured urine.
Treatment with Fluorgelb II did not result in substantially decreased PCE/TE ratios compared to the untreated controls indicating that Fluorgelb II did not have cytotoxic properties in the bone marrow. However, clinical signs after treatment and the yellow coloured urine of treated mice indicated systemic distribution and thus bioavailability of Fluorgelb II.
Biologically relevant increases in the number of PCEs with micronuclei compared to the concurrent vehicle controls were not found in bone marrow at any treatment interval and dose level. The increase in PCEs with micronuclei in males observed after 1500 mg/kg bw/day was due to a high rate of micronuclei in one mouse only. However, this value as well as the mean value for the group is within the range of the historical data, not statistically significant and, consequently, considered not biologically relevant.

Conclusion
Under the experimental conditions used, Fluorgelb II did not induce an increase in bone marrow cells with micronuclei in treated mice and, consequently, Fluorgelb II is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 28

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

Taken from SCCNFP/0689/03

Guideline: OECD n° 414
Species: Wistar rat
Route: oral
Group sizes: originally 26 mated females/group; 21-25 pregnant females/group
Material: Fluorgelb II in 0.5% sodium carboxymethylcellulose
Batch: AZ 212
Purity: > 99%
Dose levels: 0, 10, 30, 90 mg/kg bw/day in a volume of 10 ml/kg
Administration: days 6-15 of gestation
GLP: in compliance
Study date: 1991

Fluorgelb II, dissolved in 0.5% sodium carboxymethylcellulose, was administered by gavage to 4 groups of pregnant Wistar rats at dose levels of 10, 30 and 90 mg/kg bw (group sizes 22, 25 and 21, respectively). Controls received the vehicle only (group size 22). The dams were killed on day 20 of gestation. The abdominal and thoracic cavities of the dams were examined. All foetuses were examined for any external abnormalities. Half of the foetuses were examined for skeletal defects by Alizarin Red staining and the remaining foetuses were evaluated for visceral abnormalities after fixation in Bouin's fluid.

Results
Dams
No dams died during the study and no pups were delivered prematurely. Urine of animals treated with 30 and 90 mg/kg bw were stained yellow by the test material. No other treatment related effects were observed in the dams.

Foetuses
In groups treated with 30 and 90 mg/kg bw there was a slight but not statistically significant decrease of viable foetuses per female and an increase in the number of post implantation losses. No skeletal or organ deformities were reported in any of the groups.
The study authors concluded that 90 mg/kg/bw was a NOAEL for maternal toxicity, embryotoxicity and teratogenicity.

Reproductive performance: there were no significant effects on reproductive performance. Embryolethality was slightly increased at 30 and 90 mg/kg bw but the changes did not reach the level of statistical significance.

Foetuses: external foetal observation revealed no gross malformations. Skeletal observations revealed delayed ossification of metacarpals and sternum at 30 and 90 mg/kg bw. No skeletal malformations were observed. Visceral observations did not show any treatment related effects.

90 mg/kg bw was the NOAEL for maternal toxicity and teratogenicity.
In a conservative approach, the SCCNFP considers 10 mg/kg bw as the NOAEL for developmental anomalies.

Ref.: 29

3.3.9. Toxicokinetics

In vitro study with human intestinal epithelial cells

Guideline: /
Cells: Human intestinal epithelial cell line TC-7
Test substance: HC Yellow n° 13
Batch: 97/92/1094
Purity: 99.9 area% (HPLC at 254 nm)
Test concentration: 10 µM in HBSS buffer containing 1 % DMSO
Incubation time: 60 min
GLP: /

The bioavailability of HC Yellow n° 13 across the intestinal barrier was investigated in human intestinal epithelial (TC-7) cells *in vitro* in 2 independent experiments. The permeability from the apical (A, pH 6.5) to the basolateral (B, pH 7.4) side was investigated at 37 °C in 96-well transwell plates with shaking for a 60 min contact time. Analysis of the donor (apical) and receiver (basolateral) samples was done by means of HLPC-MS/MS and the apparent permeability coefficient (P_{app}) was calculated for two independent experiments. ^{14}C-mannitol (about 4 µM) was used to demonstrate the integrity of the cell monolayer. Only monolayers revealing a permeability of $< 2.5 \times 10^{-6}$ cm/sec were used. Propranolol, atenolol, vinblastine and ranitidine were analysed concurrently to demonstrate the validity of the test system.

According to the laboratory's classification system, a low permeability is considered for test items revealing a $P_{app} < 2 \times 10^{-6}$ cm/sec. A P_{app} of $2 - 20 \times 10^{-6}$ cm/sec and a $P_{app} \geq 20 \times 10^{-6}$ cm/sec classify a substance to have a moderate and a high permeability, respectively. As recommended by FDA, ranitidine (50 % absorption in humans) was used as the low permeability reference compound and propranolol (90 % absorption in humans) was used as the high permeability reference compound.

Results
The total recovery for the reference substances and HC Yellow n° 13 ranged from 73 to 162 %. The permeability figures for the reference substances propranolol (P_{app} = 58.2 and 48.0 x 10^{-6} cm/sec), a high permeability reference compound with 90 % absorption in humans, and ranitidine (P_{app} = 0.2 and <0.1 x 10^{-6} cm/sec), known to be absorbed at about 50 % in humans, were within acceptance range of 20 - 60 x 10^{-6} cm/sec and 0.0 - 2 x 10^{-6} cm/sec, respectively and demonstrated the validity of the assay.
For HC Yellow n° 13 a P_{app} of 82.6 x 10^{-6} cm/sec (86.7 and 78.5 x 10^{-6} cm/sec) was determined and thus the test substance was classified to be of high permeability, indicating a good absorption from the gastrointestinal tract.

Conclusion
A mean permeability in human intestinal epithelial (TC-7) cells of 82.6 x 10^{-6} cm/sec was obtained with HC Yellow n° 13 which classifies the test item to be of high permeability. As the absorption across the intestinal epithelium is considered to be the limiting factor of the uptake through the gastro-intestinal tract, the high permeability observed in this assay indicates a good absorption of HC Yellow n° 13 after oral administration.

Ref.: 30

Comment
The study was not performed under GLP conditions, but a statement of the quality assurance unit of the test facility is included. There is no official guideline for this assay.
However, the study was performed according to ECVAM recommendations. The generated data is considered to provide an estimation of the bioavailability of HC Yellow n° 13 after oral administration.

In vivo study in rats

Guideline:	/
Species:	Sprague Dawley Him: OFA rat
Group sizes:	3 males and 3 females
Route:	topical and oral
Material:	^{14}C- HC Yellow n° 13 at 2.5 % in formulations with and without hydrogen peroxide, and in water/DMSO (1:2)
Batch:	AZ2Ì2
Purity:	99.8 % (HPLC at 210 nm)
Application route:	Experiments **A**, **B**, **C**: dermal Experiments **D**, **E**: oral
Dose levels:	Exp. **A**: Formulation without H_2O_2: 2.5 %; 2.23 mg/cm² Exp. **B**: Formulation with H2O2; 2.5 %; 2.72 mg/cm² Exp. **C**: Solution in water/DMSO (1:2); 8.33 %; 2.85 mg/cm² Exp. **D**: solution in water/DMSO (1:2); 2.5 %; 122.2 mg/kg bw Exp, **E**: solution in water/DMSO (1:2); 2.5 %; 127.1 mg/kg bw
Treatment:	Exp. **A**, **B**, **C**: single dermal application for 30 min Exp. **D**, **E**: single oral administration (gavage)
GLP:	In compliance

*Dermal application (experiments **A**, **B** and **C**)*
^{14}C- HC Yellow n° 13 was applied dermally to groups of 3 male and 3 female rats (body weight about 200 g). The application area was 9 cm^2 and the test substance was applied at concentrations of 8.33 % in solution (water/DMSO 1:2, experiment **C**) and of 2.5 % in formulations without (experiment **A**) and with hydrogen peroxide (experiment **B**) for 30 min. The mean amount of the applied dyestuff corresponded to 9.68, 2.90 and 2.51 µg/cm^2, respectively.
After treatment the test substance was scraped off and the skin rinsed with a shampoo formulation followed by water until the rinsing water was free from colour. Rinsing solutions

were collected. During the exposure time, animals were constrained to avoid licking. After rinsing, the area was covered with gauze fixed by adhesive tape and an additional air permeable plastic cone to further prevent licking of the treated area during the 72 h in the metabolism cages.

*Oral administration (**D** and **E**):*
5 mg/kg bw (**D** and **E**) ^{14}C- HC Yellow n° 13 was administered as a 2.5 % solution in water/DMSO 2:1 by gavage to two groups of 3 male and 3 female rats each which were starved for 16 hours before treatment. In experiment **D** animals were placed in metabolism cages for 72 h. In experiment **E**, blood was taken at several time points within 24 h after administration. During the studies, blood was taken from the retrobulbar venous plexus under light ether anaesthesia. At termination of the experiment, blood samples were taken from the aorta.
Urine and faeces were collected daily (0-24, 24-48 and 48-72 h after administration) from the metabolism cages. Animals were killed 72 hours (**A**, **B**, **C**, and **D**) and 24 hours (**E**) after the application and the application sites (**A**, **B** and **C**) as well as some organs were taken and analysed for radioactivity. The radioactivity in the remaining carcass after skin removal (**A**, **B**, **C** and **D**) or removal of the gastro-intestinal tract (**D**) was also determined.

Results
Total recovery of the applied radioactivity for the individual animals in the experiments ranged from 98.0 to 99.2 % in the dermal groups and 98.73 % in the oral group **D**. The ^{14}C-content of the gastro-intestinal tract in experiment **D** was not determined.

Dermal application
Seventy two hours after application, the amount of radioactivity remaining at the application site (skin) was less than 0.15 % for all three experiments. The highest value (0.15 % of the applied dose) was noted for the formulation with hydrogen peroxide (experiment **B**). For the formulation without H_2O_2 (**A**) and with the DMSO/water solution (**C**), the figures were 0.121 and 0.146 %. The majority of the dyestuff was removed by rinsing 30 min after the application. 97.8 to 98.68 % of the applied dose was found in the washing water.
0.128 %, 0.091 % and 0.336 % of the applied doses were eliminated via urine and faeces within 72 h in experiment **A**, **B** and **C**, respectively. The lowest absorption rate was obtained for the formulation containing H_2O_2. Radioactivity was mainly excreted via urine (63 - 75 % of the amounts eliminated, equal to 62 - 74 % of the amounts absorbed) and to a lower extent via faeces (25-37 % of the amounts eliminated). 81 - 89 % of the total amount eliminated was excreted within the first 24 hours.
The radioactivity remaining in the carcass (0.0024 to 0.0033 % of the administered and 1.0 to 1.8 % of the adsorbed dose) was below or near the detection limit in experiments **A**, **B** and **C**. Residues in organs were mostly below the detection limit, with highest concentrations noted for thyroids, adrenals and fat.
Based on these results, the amount cutaneously absorbed was 0.121 % (equal to 2.90 μg/cm^2) for the formulation without H_2O_2, 0.15 % (equal to 2.50 μg/cm^2) for the formulation with H_2O_2 and 0.147 % (equal to 9.68 μg/cm^2) for the pure dyestuff in water/DMSO.

Oral application:
After oral application, ^{14}C- HC Yellow n° 13 was mainly eliminated via urine (82 % of the applied dose within 72 h) and to a minor extent via the faeces (18 %). Elimination was fast as the main part was excreted within the first 24 hours (87 %). The radioactivity found in tissues 72 h after administration was generally very low (less than 0.003 % of the applied dose per g organ), with higher values noted in fat, liver and skin. In experiment **E** the blood level reached a peak at 120 min after application and declined with an initial half-life of 1 h.

Conclusion
HC Yellow n° 13 given orally to rats is quickly absorbed and excreted within 72 h, with the majority eliminated within 24 h after application. Excretion takes place predominantly (82 %)

via urine and to a minor extent via faeces, demonstrating a good bioavailability after oral application, which is in line with findings obtained *in vitro*.
The observed excretion pattern after dermal application is similar to the one observed for the oral route, with the majority excreted via urine within 24 hours after administration. The amount cutaneously absorbed was 0.121 % (equal to 2.90 µg/cm²) for the formulation without H_2O_2, 0.15 % (equal to 2.50 µg/cm²) for the formulation with H_2O_2 and 0.147 % (equal to 9.68 µg/cm²) in water/DMSO. Low tissue residue levels were noted for both routes of exposure (dermal or oral), indicating that bio-accumulation would not be expected. The remaining radioactivity in the skin after dermal application was also low (less than 1 % of the applied dose).

Ref.: 31

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° 13

Oxidative conditions

Absorption through the skin	**A (mean + 2SD)**	**=**	**3.73 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm²**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**2.16 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.04 mg/kg bw**
NOAEL (developmental toxicity study, oral, rat)		**=**	**10 mg/kg bw**

MOS	**=**	**277**

Non-oxidative conditions

Absorption through the skin	**A (mean + 2 SD)**	**=**	**3.13 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**1.82 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.03 mg/kg bw**
NOAEL (developmental toxicity study, oral, rat)		**=**	**10 mg/kg bw/d**
MOS		**=**	**331**

3.3.14. Discussion

Physico-chemical specification
HC Yellow n° 13 is used as a hair colouring agent ("Direct Dye") in non-oxidative and in oxidative hair dye formulations at a maximum on-head concentration of 2.5%. The stability of HC Yellow n° 13 in the presence of hydrogen peroxide during a 45' period was demonstrated. The stability of HC Yellow n° 13 in typical hair dye formulations has not been demonstrated. HC Yellow n° 13 is a secondary amine, and thus, it is prone to nitrosation. Nitrosamine content in HC Yellow n° 13 has not been reported. It should not contain more than 50 ppb nitrosamine and it should not be used in the presence of nitrosating agent.

General toxicity
HC Yellow n° 13 was found to be of low acute oral and dermal toxicity in rats. In a 90-day oral toxicity study in rats, slight pancreatic islet cell degeneration, accompanied by inflammation or fibrosis of the endocrine pancreas was observed in two of the male rats treated with 90 mg/kg bw. These changes were accompanied by a high but not statistically significant blood glucose level. 30 mg/kg bw/d was considered the NOAEL. However, the NOAEL was adjusted to 21 mg/kg bw/day because of only 5 days treatment per week.
In a rat teratogenicity study, no structural abnormalities were observed in the foetuses. however, there were slight indications of developmental retardations (delayed ossification of metacarpals and sternum) following administration of 30 and 90 mg/kg bw/day during the critical days of organogenesis. While the dose of 90 mg/kg bw/d (the highest dose investigated) was judged the NOAEL for maternal toxicity and teratogenicity, the SCCS in a conservative approach considers 10 mg/kg bw/d as the NOAEL for developmental anomalies.

Irritation, sensitisation
A 5% solution of the test material was not irritant to rabbit skin.
Slight redness and chemosis of the conjuctivae was observed. According to 83/467/EEC, 5% HC Yellow n° 13 in propyleneglycol is classified as not irritant.
Mild reactions were seen with slight effects on the cornea, iris, and conjunctiva reported when tested with the undiluted substance. In all cases the mean scores were below the thresholds defined in Directive 83/467/EEC for classification as "irritant".
The sensitisation data in the dossier was generated without inducing irritation during induction.

Dermal absorption
In an *in vitro* dermal absorption assay using pig skin, the amount 2.61 ± 0.56 µg/cm² (receptor fluid + upper skin + lower skin) was considered to be bioavailable under oxidative conditions from a formulation containing 2.5% HC Yellow n° 13. As only 5 chambers were

used and the dose of formulation was too high, the mean+2SD (3.73 µg/cm²) is used in calculating the MOS.

The amount 1.95 ± 0.59 µg/cm² (receptor fluid + upper skin + lower skin) was considered to be bioavailable under non-oxidative conditions in a formulation containing 2.5% HC Yellow n° 13. As only 5 chambers were used and the dose of formulation was too high, the mean + 2SD (3.13 µg/cm²) should be used in calculating the MOS.

The study *in vivo* is discussed in the toxicokinetics section.

Toxicokinetics

In vitro study

A mean permeability in human intestinal epithelial (TC-7) cells of 82.6 x 10^{-6} cm/sec was obtained with HC Yellow n° 13 which classifies the test item to be of high permeability. As the absorption across the intestinal epithelium is considered to be the limiting factor of the uptake through the gastro-intestinal tract, the high permeability observed in this assay indicates a good absorption of HC Yellow n° 13 after oral administration.

In vivo study

HC Yellow n° 13 given orally to rats is quickly absorbed and excreted within 72 h, with the majority eliminated within 24 h after application. Excretion takes place predominantly (82 %) via urine and to a minor extent via faeces, demonstrating a good bioavailability after oral application, which is in line with findings obtained *in vitro*.

The observed excretion pattern after dermal application is similar to the one observed for the oral route, with the majority excreted via urine within 24 hours after administration. The amount cutaneously absorbed was 0.121 % (equal to 2.90 µg/cm^2) for the formulation without H_2O_2, 0.15 % (equal to 2.50 µg/cm^2) for the formulation with H_2O_2 and 0.147 % (equal to 9.68 µg/cm^2) in water/DMSO. Low tissue residue levels were noted for both routes of exposure (dermal or oral), indicating that bio-accumulation would not be expected. The remaining radioactivity in the skin after dermal application was also low (less than 1 % of the applied dose).

Mutagenicity

Overall, the genotoxicity of HC Yellow n° 13 is sufficiently investigated for the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. HC Yellow n° 13 treatment did not result in an induction of gene mutations in bacteria. In an *in vitro* chromosome aberration test, an increase in the number of cells with chromosomal aberrations was found after treatment with HC Yellow n° 13. The positive effect found in the *in vitro* chromosome aberration test could not be confirmed with an *in vivo* test. HC Yellow n° 13 did not induce an increase in bone marrow cells with micronuclei in an *in vivo* micronucleus test.
As the positive effect found *in vitro* was not confirmed in an *in vivo* test, HC Yellow n° 13 itself 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° 13 as a direct dye with a maximum on-head concentration of 2.5% in oxidative and non-oxidative hair dye formulations does not pose a risk to the health of the consumer.

A possible sensitising potential of HC Yellow n° 13 cannot be excluded.

HC Yellow n° 13 is a secondary amine, and thus, it is prone to nitrosation. Nitrosamine content in HC Yellow n° 13 has not been reported. It should not contain more than 50 ppb nitrosamine and it should not be used in the presence of nitrosating agent.

5. MINORITY OPINION

Not applicable

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