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

CONCERNING

HC YELLOW N° 13

COLIPA n° B102

adopted by the SCCNFP during the 24th plenary meeting
of 24-25 June 2003

1. Terms of Reference

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is HC Yellow n° 13 for use in cosmetic products?

* Does the SCCNFP propose any restrictions or conditions for its use in cosmetic products?

1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.
The extent to which these validated methods are applicable to cosmetic products and its ingredients is a matter of the SCCNFP.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. Toxicological Evaluation and Characterisation

2.1. General

2.1.1. Primary name

HC Yellow n° 13 (INCI)

2.1.2. Chemical names

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

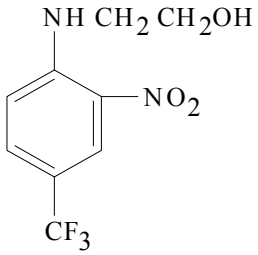
2.1.3. Trade names and abbreviations

Fluorgelb II
Cos 218
COLIPA n° B102

2.1.4. CAS/EINECS no.

CAS n° : 10442-83-8
EINECS n° : /

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_9H_9F_3N_2O_3$
Mol weight : 250.2

2.1.7. Purity, composition and substance codes

4 batches have been tested. Purity > 99 % (HPLC, ^{1}H-NMR/^{19}F-NMR, IR)

Water content : < 0.06 %
Loss on drying : < 0.12 %
Total impurities : 0.01 – 0.34 %

3 trace substances have been found by HPLC. However, their identification and characterisation was not done.

2.1.8. Physical properties

Appearance	:	yellow crystalline powder
Melting point	:	/
Boiling point	:	/
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{ow}	:	/

2.1.9. Solubility

Water	:	0.16%
Propylene glycol	:	> 20%

General comments on analytical and physico-chemical characterisation

* Relevant physico-chemical parameters are not given.
* 4 batches of HC Yellow n° 13 have been analysed for purity. 1 of these has been used in the studies.
* 3 trace impurities have been found.
* The data on the stability of HC Yellow n° 13 is insufficient.
* HC Yellow No. 13 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.

2.2. Function and uses

HC Yellow n° 13 is used in oxidative hair dye formulations at a maximum concentration of 5.0%. Since oxidative hair dye formulations are mixed with hydrogen peroxide before application, the in-use concentration is 2.5%. In colour setting lotions, the maximum concentration is 5.0%.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Guideline	:	OECD 401 (1987)
Species	:	Wistar rats, Crl: (WI)BR
Group size	:	5 males + 5 females
Material	:	Fluorgelb II in 2% gum arabic
Batch no	:	AR 902
Purity	:	99.7 %
Dose	:	2000 mg/kg bw in a volume of 10 ml/kg
Observation period	:	14 days
GLP	:	in compliance

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 LD_{50} was greater than 2000 mg/kg bw.
Ref. : 1

2.3.2. Acute dermal toxicity

Guideline	:	OECD 402 (1987)
Species	:	Sprague Dawley rats, Him:OFA
Group size	:	5 males + 5 females
Material	:	Fluorgelb II moistened with distilled water
Batch no	:	AR 902
Purity	:	99.7 %
Dose	:	2000 mg/kg bw on an area of 5 x 6 cm
Observation period	:	14 days
GLP	:	in compliance

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 LD_{50} was reported to be greater than 2000 mg/kg bw.

Ref. : 2

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

No data

2.3.5. Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Sub-chronic oral toxicity

Guideline	:	OECD n° 408
Species	:	Wistar rats, Crl: (WI) BR
Group sizes dose	:	15 males and 15 females (+ 10 males and 10 females for recovery high- and control group)
Material	:	Fluorgelb II in 0.5% aqueous sodium carboxymethylcellulose
Batch no	:	AZ 212
Purity	:	> 99 %
Dose levels	:	0, 10, 30 and 90 mg/kg body weight in a volume of 10 ml/kg
Exposure	:	5 days per week for 90-92 days
GLP	:	in compliance

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. : 9

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No data

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Test on diluted material

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 no	:	AR 902
Purity	:	99.7 %
Dose	:	0.5 ml on 6 cm^2
GLP	:	in compliance

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. : 5

Test on undiluted material

Guideline	:	OECD n° 404
Species	:	New Zealand white rabbit
Group size	:	3 females

Material	:	Fluorgelb II
Batch no	:	AR 902
Purity	:	99.7 %
Dose	:	0.5 g on a cellulose patch moistened with 1 ml distilled water
GLP	:	in compliance

A cellulose patch soaked with 1 ml distilled water was covered with 0.5g of the undiluted test material and applied to the shaven backs of 3 rabbits (closed patch) for 4 hours. Material residues were then wiped off using wetted cellulose tissue. Skin reactions were read 1, 24, 48 and 72 hours after patch removal. Additional examinations of the skin were performed 6, 8, 10, 13, 15, 17 and 21 days after the end of exposure.

Results
No signs of erythema or oedema were observed. The material was “non-irritant” to rabbit skin.

Ref. : 6

2.4.2. Irritation (mucous membranes)

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 no	:	AR 902
Purity	:	99.7 %
Dose	:	0.1 ml
GLP	:	in compliance

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 is classified as not irritant.

Ref. : 3

Test on undiluted material

Guideline	:	OECD n° 405
Species	:	rabbit (New Zealand white)
Route	:	eye
Group size	:	3 females

Material	:	Fluorgelb II
Batch no	:	AR 902
Purity	:	99.7 %
Dose	:	Approximately 0.1 ml
GLP	:	in compliance

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. : 4

2.5. Sensitisation

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 no	:	AR 902		
Purity	:	99.7 %		
Concentrations used	:	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		

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. The test material was non-sensitising.

Remark
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.
Ref. : 7

2.6. Teratogenicity

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 no	:	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

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. : 13

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Guideline	:	/
Species	:	Sprague Dawley Him: OFA rat
Group size	:	3 male and 3 female
Route	:	topical
Material	:	^{14}C-Fluorgelb II at 2.5% in formulations with and without hydrogen peroxide, and in water/DMSO (1:2)
Batch no	:	AZ 212 (purity 98%)
Dose level	:	Approximately 1g for each formulation or solution
GLP	:	in compliance

^{14}C-labelled Fluorgelb II, included in two different hair dye formulations (at 2.5%) with and without hydrogen peroxide, and in an aqueous DMSO solution (at 8.33 %) was applied to the clipped dorsal skin (3x3 cm) of the rats for 30 minutes and then washed off.. Oral administration of the test material (2.5% solution) was used as a reference. Radioactivity of rinsings, application site, urine, faeces, blood, organs and carcass was estimated by liquid scintillation counting. Percutaneous absorption was calculated from the amount of ^{14}C eliminated from the body (72 hours post application) plus the amount still present in the carcass compared with the applied dose.

Results
The mean percutaneous absorption was 2.9 µg/cm^2 for the 2.5% hair dye formulation without hydrogen peroxide, 2.5 µg/cm^2 for the 2.5% formulation with hydrogen peroxide and 9.69 µg/cm^2 for the 8.33 % solution in DMSO.

From this study the SCCNFP concludes :

* For oxidative hair dyes (max. in-use concentration 2.5%) a percutaneous absorption of 2.5 µg/cm^2 can be assumed;

* For use in colour setting lotions, the study was conducted with a too low concentration (2.5% instead of in-use concentration of 5%). Therefore the absorption obtained with a 8.33 % solution in DMSO/water (viz. 9.69 µg/cm^2) is taken as a worst case.

Ref. : 8

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline	:	/
Species/strain	:	*S. typhimurium,* TA97, TA98 and TA100

Replicates	:	Triplicate plates, 2 independent tests
Test substance	:	Fluorgelb II in DMSO solution
Batch no	:	AR 822
Purity	:	not stated
Concentrations	:	5 concentrations with and without metabolic activation

Assay # 1
TA 98 : 1, 10, 100, 1000, 10000 μg/plate
TA 97 : 1, 10, 100, 1000, 6000 μg/plate
TA 100 : 1, 10, 100, 1000, 6000 μg/plate

Assay # 2
TA 98 : 3, 10, 30, 100, 300, 1000, 3000, 6000 μg/plate
TA 97 : 3, 10, 30, 100, 300, 1000, 3000 μg/plate
TA 100 : 3, 10, 30, 100, 300, 1000, 3000 μg/plate

GLP : in compliance

HC Yellow n° 13 has been investigated for gene mutation 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. Negative and positive controls were in accordance with the OECD guideline.
Toxicity was seen in at 1000 μg/plate.

Results
With or Without S9 mix : no dose related or biologically relevant increase in revertant numbers was observed, in any of the 3 *S. typhimurium* tester strains.

Conclusions
Based on the reversion rate, and under the conditions of the assays performed, it is concluded that the test agent Fluorgelb II (batch AR 822 - purity not stated)is negative in the *S. typhimurium* tester strains in the absence or in the presence of S9 mix.
However, the test is unsuitable for genotoxicity evaluation for the following reasons : only 3 tester strains has been evaluated while guidelines recommend tester strains that cover both A-T and G-C damage types.

Ref. : 10

Bacterial Reverse Mutation Test

Guideline	:	OECD 471, EC B14.
Species/strain	:	*S. typhimurium,* TA98, TA100, TA1535, TA1537, TA 1538
Replicates	:	Triplicate plates, 2 independent tests
Test substance	:	C000218 dissolved in DMSO
Batch no	:	97/92/1094
Purity	:	99.9 % (GC/HPLC)
Concentrations	:	5 concentrations – direct plate incorporation assay, with and without metabolic activation

		Test #1 : 1, 10, 100, 1000 and 5000 µg/plate
		Test #2 : 30, 100, 300, 1000 and 3000 µg/plate
GLP	:	in compliance

HC Yellow n° 13 has been investigated for gene mutation 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. Negative and positive controls are in accordance with the OECD guideline.

Results
Toxicity
A toxicity as evidenced by a reduction in the number of spontaneous revertants per plate and a abnormal background lawn was observed – both with or without S9 and for every tester strains – at dose levels of 3000 and 5000 µg/plate.
With or Without S9 mix : no dose related or biologically relevant increase in revertant numbers was observed, in any of the *S. typhimurium* tester strains and in any of the experiments performed.

Conclusions
The test is acceptable for evaluation.
Based on the reversion rate, and under the conditions of the assays performed, it is concluded that the test agent C000218 dissolved in DMSO is negative in any of the *S. typhimurium* tester strains in the absence or the presence of S9 mix.

Ref. : 14

***In vitro* Mammalian Chromosomal Aberration Test, study 1**

Guideline	:	/
Species/strain	:	Chinese Hamster Ovary Cells
Replicates	:	Duplicate cultures but no independent experiments
Test substance	:	Fluorgelb II in DMSO solution
Batch no	:	not stated
Purity	:	not stated
Concentrations	:	Preliminary dose range finding study : 0.06, 0.18, 0.55, 1.64, 4.94, 14.8, 44.4, 133.3 and 400 µg/ml
		Test without S9 : 0.18, 4.94 and 133.3 µg/ml
		Test with S9 : 1.64, 14.8 and 133.3 µg/ml
GLP	:	not in compliance

HC Yellow n° 13 has been investigated for induction of chromosomal aberrations in CHO cells.
The test concentrations were established from a preliminary toxicity study.
Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system.

Exposure and concentrations
samples were cultured during 24 h and then exposed during 2 hours With or Without S9.
Cultures were prolonged during 22 hours before harvest.

Results
Toxicity
Relevant toxic effects as evidenced by a decrease in Mitotic Index (MI) was observed in the absence or in the presence of S9 mix in from 133.3 µg/ml.

Structural chromosome aberrations
With or Without S9 mix : no statistically and biologically significant increase in the aberration rate was observed as compared to the corresponding solvent control.

Polyploidy
Not considered

Conclusions
There are no indication of clastogenicity. However, according to the modern standard strategies and guidelines, the assay is unsuitable for evaluation. There are no independent repeat study, the exposure and expression period are inadequately selected, test substance is not characterised, batch and purity are not given.

Ref. : 11

***In vitro* Mammalian Chromosomal Aberration Test, study 2**

Guideline	:	OECD 473, EC B10.
Species/strain	:	Chinese Hamster V79 Cells
Replicates	:	Duplicate cultures
Test substance	:	Fluorgelb II, 89105 in DMSO
Batch no	:	CH 97 / 92 / 1094
Purity	:	99.7 %
Concentrations	:	25 - 250 µg/ml with and without metabolic activation.
GLP	:	in compliance

HC Yellow n° 13 has been investigated for induction of chromosomal aberrations in chinese hamster V79 cells. Liver S9 fraction from rats induced with β-naphthoflavone/phenobarbitone was used as the exogenous metabolic activation system. The test concentrations were established from a preliminary toxicity study. With respect to the molecular weight of Fluorgelb II , the maximum concentration tested was 2600 µg/ml (that correspond to ± 10 mM) have been selected.

	Exposure period	Recovery	Preparation interval	Doses µg/ml		
without S9 mix:	4 hours	14 hours	18 hours	50	100	150
with S9 mix :	4 hours	14 hours	18 hours	50	100	150

Results
pH and Osmolarity
In the range finder study, no precipitation occurred

At the top concentration, no influence of the pH or osmolarity was noted (solvent ctrl pH: 7.2; 391 mOsm /top dose of 2600 µg/ml pH : 7.3; 369 mOsm).

Toxicity
Toxic effects as evidenced by a reduction in cell numbers was observed in the presence of S9 mix at 150 µg/ml.
No clear reduction of the mitotic index was noted in the absence of activation. With S9, at the top dose the mitotic index was 73.5 % of the control.

Structural chromosome aberrations

* without activation system.
No statistically significant or biologically relevant increase in the number of cells with structural chromosomal aberrations was noted.

* with activation system.
A statistically and/or biologically significant dose-dependent relevant increase in the number of aberrant cells was observed as compared to the corresponding solvent control.
50 µg/ml 0.5 %
100 µg/ml 5.5 %
150 µg/ml 15.0 %
Moreover, many metaphase cells displayed "exchange" figures that are considered as indicators of clastogenicity.

Polyploidy
Taken into account that no specific positive control agent has been used in this assay, and that only metaphases with 22 ± 1 chromosomes were considered for scoring, polyploidy means a near tetraploid karyotype.
No biologically relevant increase in the number of polyploid metaphases was recorded.

The assay is acceptable for evaluation
The test substance is considered positive for clastogenic activity in Chinese hamster V79 cell line in the presence of activation under the conditions of this test.

Ref : 15

2.8.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline	:	/
Species	:	Crl:NMRI BR mouse
Group sizes	:	5 males and 5 females
Test substance	:	Fluorgelb II dissolved in DMSO
Batch no	:	AR 822
Purity	:	not stated
Dose levels	:	0 and 1500 mg/kg bw
Administration	:	gavage

Sacrifice times : after 24, 48 and 72 hours.
GLP : in compliance

HC Yellow n° 13 has been investigated for induction of micronuclei in the bone marrow cells of male or female mice. Dose levels were determined on the basis of the results of a preliminary dose-range finding study. Negative and positive controls were in accordance with the OECD guideline.

Test doses : Fluorgelb II dissolved in DMSO was administered by 1 single oral dose. 3 sacrifice times were selected : 24 h, 48 h and 72 h after oral administration. Bone marrow smears were obtained from the positive control group 24 after dosing.

Number of cells scored : A total of at least 1000 erythrocytes were examined from each animal ; the incidence of micronucleated erythrocytes and the ratio of polychromatic erythrocytes to normochromatic erythrocytes were calculated.

Results
Reactions to treatment
No toxic effects were observed but the animals appeared very weak at the dose of 2000 µg/ml. Therefore, 1500 µg/kg bw was selected as the top dose.

Mean values of micronucleated PCE
No statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic cells over the concurrent vehicle control values were observed at any sampling times.

PCE/NCE ratio
The ratio is not presented as such.
The proportion of nucleated cells was significantly lower in the dosed group 72 hours post dosage as compared with negative controls. This value was also outside of the historical negative control.

Conclusions
Though not conforming to OECD guidelines, the test has been acceptable for evaluation taken as such. Fluorgelb II (purity not stated) gave negative results in the mammalian erythrocyte micronucleus test. However, the study did not demonstrate that bone marrow was reached by the test agent.

Ref. : 12

2.11. Safety evaluation

NOT APPLICABLE

2.12. Conclusions

HC Yellow n° 13 consists for more than 99 % of N-(2-hydroxyethyl)-2-nitro-4-trifluormethyl-aniline. 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 ^{14}C-labelled a.i.) indicated a maximum penetration of 2.5 μg/cm^2 for oxidative hair dyes (max. in-use concentration 2.5%) , and of 9.69 μg/cm^2 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.

2.13. References

1. Lindena, J. and Oetjen, M. “Acute oral toxicity test of Fluorgelb II in rats” IBR Foschungs GmbH Walsrode, 27.06.1990
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3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the information submitted is inadequate to assess the safe use of the substance.

Before further consideration, the following information is required :

* Relevant physico-chemical data, as well as data on nitrosamine content of the dye and in hair dye formulations;

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