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

CONCERNING

BASIC YELLOW 57

COLIPA n° C10

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 Basic Yellow 57 safe 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

The dossier of Basic Yellow 57 has been presented in two separate submissions. The first submission included all required studies for the safety evaluation. Additional (new) data on percutaneous absorption and mutagenicity testing of Basic Yellow 57 were presented in the second submission.

According to the first submission, Basic Yellow 57 (CAS no. 68391-31-1) is a mixture of 63.5% dye (5-hydroxy-3-methyl-1-phenyl-4-(3'-trimethylammoniophenylazo)-pyrazole, chloride) with 9.6% sugar (undefined), 6.9% volatile matter and 20% inorganic salts (chloride, sulphate etc.). Data (including percutaneous absorption), teratogenicity and mutagenicity/genotoxicity were generated using different batches (of unknown purity) of this preparation.

The second submission describes the Basic Yellow 57 (CAS 68391-31-1) as >98.5% dye (5-hydroxy-3-methyl-1-phenyl-4-(3'-trimethylammoniophenylazo)-pyrazole, chloride). This material is used for additional experiments: percutaneous absorption and genotoxicity/mutagenicity testing.

The SCCNFP requires a complete clarification on the identification and purity of the test materials used in the two sets of experiments. This should also include the certificates of analysis.

As the information on the purity/composition of the Basic Yellow 57 used in the two sets of data are different from each other, it is not possible for the SCCNFP to perform the corresponding safety assessment requested.

2.1. General

2.1.1. Primary name

Basic Yellow 57 (INCI name)

2.1.2. Chemical names

5-Hydroxy-3-methyl-1-phenyl-4-(3'-trimethylammoniophenylazo)-pyrazol, chloride

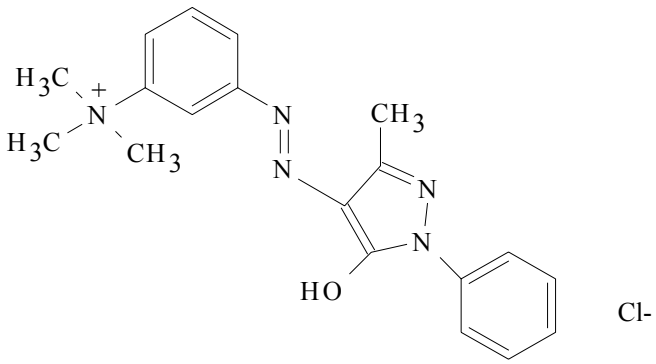
2.1.3. Trade names and abbreviations

Arianor Straw Yellow
CI n° 12719
COLIPA n° C10

2.1.4. CAS/EINECS no.

CAS n° : 68391-31-1
EINECS n° : 269-943-5

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{19}H_{22}N_5OCl$
Mol weight : 371.5 (as chloride)

2.1.7. Purity, composition and substance codes

Composition :	Dye (as chloride)	63.5%
	Sugar	9.6%
	volatile matter/water of crystallisation	6.9%
	inorganic salts (chloride, sulphate, etc.)	to 100%

Batch : RS 68216101
Purity : Determined by HPLC > 98.9 % (AUC, HPLC), UV detection at 246 nm

2.1.8. Physical properties

Appearance : yellowish orange powder, odourless
Melting point : 140-160 °C (decomposition)
Boiling point : /
Density : /
Rel. vap. dens. : /
Vapour Press. : /
Log P_{ow} : /

2.1.9. Solubility

Water : soluble
Alcohol : soluble

2.2. Function and uses

Basic Yellow 57 is used in direct hair dye formulations in concentrations up to 2.0%. It is common practice for 35 ml of undiluted formulation to be applied for a period of 30 minutes before washing.

General comments on analytical and physico-chemical characterisation

* Basic Yellow 57 is not a single compound, but a mixture of a dye with other chemicals. Consequently the chemical name presented for Basic Yellow 57 is not correct. All tests except "percutaneous absorption study" and "In vitro mammalian chromosomal aberration test" reported in Submission II are performed using the dye preparation containing approximately 63.5% 5-Hydroxy-3-methyl-1-phenyl-4-(3'-trimethylammoniophenylazo)-pyrazol, chloride.
* The UV spectrum of Basic Yellow 57 shows significant absorbance at 246nm and 383 nm, with λ_{max} at 383 nm. The chromatographic purity has been described at 246 nm, but not at 383 nm.
* Basic Yellow 57 is a mixture of the dye with 9.6% sugar and > 20% inorganic salts. The chemical specifications of the sugar and inorganic salts are not reported.
* The dye contains 6.9% volatile matter/water of crystallisation. The information is insufficient as the content of volatile organic solvents is not given.
* Density and Log P_{ow} of the dye has not been reported.
* Quantitative data on solubility of Basic Yellow 57 are not reported.
* No information is provided on the stability of the dye in test solutions as well as in hair dye formulations.
* The purity of the dye used in several tests has not been described.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Study 1

Guideline	:	/
Species	:	CFY rat
Group size	:	2 males + 2 females
Material	:	Arianor Straw Yellow in 1% aqueous methylcellulose
Batch no	:	KS 3131
Dose	:	0, 0.1, 1.0, 2.0 and 4.0 g/kg bw in volumes of 1.0 to 40 ml/kg
Observation period	:	14 days
GLP	:	Not in compliance

Groups of 2 male and 2 female rats received a single oral dose of 0.1, 1.0, 2.0 and 4.0 g/kg bw. Control animals received 1% aqueous methylcellulose in a volume of 40 ml/kg. The animals were observed daily for 14 days for clinical abnormalities and mortality. Body weights and macroscopic observations were recorded, but histological examinations were not performed.

Results
One male and two female rats died after a dose of 2.0 g/kg bw. All animals treated at 4.0 g/kg bw died within one week of dosing. There were no mortalities at lower doses. Signs of reaction to treatment, observed shortly after dosing, included piloerection and abnormal body carriage (hunched posture), which were accompanied by lethargy and diarrhoea in rats treated with doses greater than 0.1 g/kg, and decreased respiratory rate, pallor of the extremities and ptosis at 1.0 g/kg and by increased salivation and diuresis in rats treated with 2.0 g/kg. The LD_{50} was reported to be between 1000 and 2000 mg/kg bw.

Ref. : 1

Study 2

Guideline	:	/
Species	:	Sprague Dawley CD rat
Group size	:	5 males + 5 females
Material	:	Arianor Straw Yellow in distilled water
Batch no	:	not stated
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 mortality and clinical abnormalities. Body weights and macroscopic observations were recorded, but histological examinations were not performed.

Results
No mortalities were reported. Clinical signs of reaction to treatment observed shortly after dosing in all rats were piloerection, abnormal body carriage (hunched posture), abnormal gait (waddling) and increased salivation. The bodyweights were reported to be normal for the strain, but there was no control group for comparison. The LD_{50} was reported to be greater than 2000 mg/kg bw.

Ref. : 2

Study 3

Guideline	:	/
Species	:	CF1 mouse
Group size	:	10 males
Material	:	Arianor Straw Yellow in olive oil
Batch no	:	not stated
Dose	:	0.631, 1.0, 2.51 and 5.01 g/kg bw in a volume of 20 ml/kg
Observation period	:	7 days

GLP : Not in compliance

Arianor Straw Yellow was administered to groups of 10 male CF1 mice at dose levels of 0.631, 1.0, 2.51 and 5.01 g/kg bw. Animals were observed for a period of 7 days.

Results
Signs of reaction to treatment were an increased respiratory rate, and tremors. The general condition of the mice deteriorated at the higher dose levels. The LD50 was reported to be 2350 mg/kg bw.

Ref. : 3

2.3.2. Acute dermal toxicity

No data

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

Study 1

Guideline	:	/
Species	:	Wistar MuRa Han 67 SPF
Route	:	oral
Group size	:	20 males and 20 females
Material	:	commercial grade Arianor Straw Yellow in aqueous solution
Batch no	:	not stated
Dose levels	:	0 and 50 mg/kg bw in a volume of 10 ml/kg
Exposure	:	5 days per week for 12 weeks
GLP	:	not in compliance

Commercial Arianor Straw Yellow, in aqueous solution, was administered by oral gavage daily, 5 days per week for 12 weeks, to groups of 20 male and female Wistar rats at 50 mg/kg bw. The controls were treated with the vehicle alone.

All animals were observed daily for clinical signs and mortality. Body weight and food consumption were recorded at weekly intervals. Haematological, clinical chemistry and urine-analyses were performed. At autopsy, organ weights were recorded and the main organs were examined macroscopically and histologically.

Results
No adverse effects or mortalities occurred. The urine excreted by the treated animals was slightly coloured. There was a small but significant reduction (less than 5%) in the body weight gains of female rats following administration for 4 weeks, 5 weeks, 6 weeks and at termination (12 weeks). Haematological analyses showed an increase in both the mean cell volume and haematocrit of treated male rats. No treatment-related effects were noted in female rats. Biochemical and urine-analyses gave no clear evidence of treatment-related effects. Macroscopic observations and histopathology revealed no differences between control and treated animals. The dose level of 50 mg/kg bw/day was considered to be on the borderline of toxicity.

Ref. : 8

Study 2

Guideline	:	/
Species	:	Sprague Dawley CD
Route	:	oral
Group size	:	10 males and 10 females
Material	:	commercial grade Arianor Straw Yellow in aqueous solution
Batch no	:	KS 1823
Purity	:	/
Dose levels	:	0 and 20 mg/kg bw in a volume of 10 ml/kg
Exposure	:	5 days per week for 13 weeks
GLP	:	In compliance

Commercial Arianor Straw Yellow, in aqueous solution, was administered by oral gavage daily, 5 days per week for 13 weeks, to groups of 10 male and female Sprague Dawley rats at 20 mg/kg bw. The controls received the vehicle alone.
All animals were observed daily for clinical signs and mortality. Body weight and food consumption were recorded at weekly intervals. Haematological, clinical chemistry and urine-analyses were performed. Opthalmological examinations were performed at the start and end of the study. At autopsy, organ weights were recorded and the main organs were examined macroscopically and histologically.

Results
No mortalities occurred. The body weight gain of treated animals was comparable to the control group. There were no indications of treatment-related effects from clinical, macroscopic or microscopic examinations. The dose of 20 mg/kg body weight/day was considered to be the 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 undiluted material

Guideline	:	/
Species	:	New Zealand white rabbit
Route	:	skin
Group size	:	3 males and 3 females
Material	:	undiluted Arianor Straw Yellow
Batch no	:	not stated
Dose	:	0.5 g/in^2
GLP	:	Not in compliance

Undiluted Arianor Straw Yellow was applied at the level of 0.5 g/in^2 to the backs of 3 rabbits of each sex with shorn intact or scarified skin. The sample was occlusively covered and left in place for 24 hours. Readings were made according to Draize upon removal of the test material and daily for 14 days post administration.

Results
There were no observable reactions to the dye. Arianor Straw Yellow was considered “not irritant” to rabbit skin.

Ref. : 5

Test on diluted material

Guideline	:	/
Species	:	New Zealand white rabbit
Route	:	skin
Group size	:	3 (sex not specified)
Material	:	Arianor Straw Yellow moistened 1:1 with distilled water
Batch no	:	9-1398 K
Purity	:	/
Dose	:	0.5 g
GLP	:	Not in compliance

0.5 g of the test material was dampened with 0.5 ml distilled water and applied to an area of 1 in^2 on the backs of 3 rabbits each with shorn intact or scarified skin. The sample was covered by an

impervious material and left in place for 24 hours. Skin reactions were recorded after 24 and 72 hours.

Results
There were no observable reactions to the dye. Arianor Straw Yellow was considered as “not irritant” to rabbit skin.

Ref. : 6

2.4.2. Irritation (mucous membranes)

Guideline	:	/
Species	:	New Zealand white rabbit
Route	:	eye
Group size	:	3 (sex not specified)
Material	:	0.5% Arianor Straw Yellow solution in physiological saline
Batch no	:	not stated
Dose	:	0.1 ml
GLP	:	not in compliance

0.1 ml of 0.5% solution Arianor Straw Yellow was instilled into the conjunctival sac of the left eye of three rabbits. The right eye was treated with 0.1 ml of the vehicle and served as a control. Eye reactions were recorded at 30 and 60 minutes and 1 and 2 days following and evaluated by the Draize method.

Results
The treatment provoked no effects on the cornea or iris in any of the test animals, however, there was a discoloration of the conjunctivae.
This test was conducted at a concentration below the intended in-use maximum of 2%. A mild reaction could have been masked by the discoloration caused by the dye.

Ref. : 4

2.5. Sensitisation

Guideline	:	OECD n° 406		
Species	:	Hartley/Dunkin guinea pigs		
Group size	:	10 female		
Material	:	Arianor Straw Yellow in aqueous solution		
Batch no	:	9-1398 K		
Concentrations used	:	intradermal induction	:	0.1%
		topical induction	:	75%
		challenge	:	25% and 5%
GLP	:	not in compliance		

Arianor Straw Yellow was prepared as a 0.1% w/v solution in water (injection 1). Freund’s complete adjuvant was diluted with an equal volume of water (injection 2). A 1:1 mixture of the material solution and Freund’s complete adjuvant solution was prepared (injection 3). The induction of sensitisation was made through 3 pairs of 2 intradermal injections. One week after the injections a solution of 75% w/v of the material in distilled water was topically applied. The

animals were challenged topically two weeks after the induction period using Arianor Straw Yellow 25% w/v followed by a further challenge one week later with 5% w/v in distilled water. Potential skin reactions were read 24, 48 and 72 hours after the final challenge.

Results
The intradermal injection caused an irritation response, that was still present at the time of the topical induction. Following the first challenge, erythema was seen in 7 of the 10 animals. The subsequent challenge with 5% Arianor Straw Yellow was therefore applied to determine whether the response was due to irritation or sensitisation. Erythema was observed in 2 of the animals at 24 hours, but had resolved by 48 hours.

Ref. : 7

2.6. Teratogenicity

Guideline	:	/
Species	:	Sprague Dawley rat, CD strain
Route	:	oral
Group size	:	control group: 20; test group: 23
Material	:	Arianor Straw Yellow in distilled water
Batch no	:	KS 1823 (purity not stated)
Dose levels	:	0 and 50 mg/kg bw/day in a volume of 10 ml/kg
Administration	:	days 6-15 of gestation
GLP	:	In compliance

Arianor Straw Yellow was administered by gavage daily to 23 pregnant rats at a dose 50 mg/kg bw/day from days 6 to 15 of gestation. Twenty control animals were given the vehicle alone (distilled water). The dams were observed twice daily and weighed daily. On day 20 post coitum the dams were sacrificed and Caesarean sections were performed. The number of implantation sites, resorptions, living foetuses and the number of corpora lutea were counted in each litter. The weight of placenta, uterus, foetuses and the sex of the foetuses were recorded. About one-third of each litter was prepared and examined for soft tissue anomalies. The remaining foetuses were examined for skeletal abnormalities after staining with alizarin red S.

Results
Dams : there were no mortalities, abortions or changes in mean body weight gain in dams treated with Arianor Straw Yellow.
Foetuses : there were no treatment related effects on reproduction data or malformations of the foetuses. The level of skeletal variation or ossification in the test and control group was regarded to be similar.
The test material produced no indications of maternal toxicity, embryo-toxicity or teratogenicity under the test conditions employed at the dose of 50 mg/kg bw/day.

Ref. : 10

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Human study

Method	:	human volunteer study
Group size	:	10 males
Material	:	1 mM Arianor Straw Yellow in 40% aqueous isopropanol
Batch no	:	not stated
Applic levels	:	20µl on 5.3 cm² skin of the inner forearm
GLP	:	not in compliance

20µl of a 1 mM solution of the test material, in 40% aqueous isopropanol, were applied to five separate skin areas (5.3 cm² s/c) of the inner forearm. After 10 minutes, and 24, 48 and 72 hours, the dye stains of one treatment area after the other were removed by ten repeated strippings with Tesafilm-Spezial^{R} tape. During the intervals between sampling the skin areas were protected by a special non-occlusive mould. The stripping-tapes were glued on to a white cardboard and kept in the dark until they were analysed. The amounts of the dye that possibly penetrated the skin were estimated from the recovery rates. The normal degradation of the dye during the 72 hours time of investigation caused by heat, humidity and the microbial flora of the skin was taken into account.

Results
It was reported that the dye diffused only to a minor degree into the horny layer; according to the corrected recovery rates. It was concluded that Arianor Straw Yellow was not absorbed by the skin.
The study is inadequate and unsuitable for evaluation as no quantitative data is reported.

Ref. : 15, 16

In vivo study of percutaneous absorption in rats

Guideline	:	/
Species	:	Sprague Dawley rat
Group size	:	3 (sex not specified)
Route	:	topical
Material	:	^{14}C-Arianor Straw Yellow in a setting lotion formulation
Batch no	:	not stated
Dose levels	:	200 mg formulation
GLP	:	not in compliance

200 mg of a hair setting lotion formulation containing 2.592 µCi ^{14}C-labelled Arianor Straw Yellow, was applied to the clipped dorsal skin of the rats. From the information provided the formulation appears to have contained about 0.1% Arianor Straw Yellow. The animals were lightly anaesthetised for the first hour, after which they were fitted with collars to prevent licking of the application site. Excretion of radiocactivity via urine and faeces was measured for 24 hours after application.

Results
The recoveries of radioactivity in urine and faeces from two rats were very low: less than 0.1% of the applied radioactivity in the faeces and less than 0.3% in the urine. One animal excreted more

than 2.3% of the applied radioactivity in the urine and 0.01% in the faeces. The study is inconclusive.

Ref. : 17

***In vitro* study of percutaneous absorption**

Guideline	:	draft OECD 428
Tissue	:	Pig skin, dermatomed (exposure area: 2.54 cm²)
Method	:	Franz diffusion cells
Test Material	:	Basic Yellow 57, 2% dye in an aqueous solution or in a standard formulation
Batch No	:	RS 68216101
Purity	:	> 99 %
Dose levels	:	- 10 µl/cm² of the aqueous solution. 197 µg/cm² of test material. - 10 mg/cm² of the formulation. 180 µg/cm² of test material.
Receptor fluid	:	Physiological saline containing 25 % ethanol.
Replicate cells	:	6 for each formulation
Analyt. method	:	HPLC methodology (UV detection at 382 nm) Quantitation limit between 0.05 µg/ml (receptor fluid) and 0.005 µg/ml (all other samples)
GLP	:	in compliance

The skin penetration of Basic Yellow 57 was evaluated in a static Franz diffusion cell system using pig skin dermatomed at 400 µm. The integrity of the skin was checked by TER (Transdermal electrical resistance). Basic Yellow 57 was adequately soluble in physiological saline alone, the ethanolic receptor fluid was chosen as it reduced interferences during analysis but without influence the integrity of the membrane or the rate of penetration.

Two skin percutaneous studies were performed: with an aqueous solution of the dye and with a formulation containing the dye both at a 2% concentration of the test material. After the application of the samples on the skin surface for 30 min, the skin surface excess was washed off with physiological saline and left unoccluded for the entire 48h exposures period. The content of dye was determined by HPLC in the following compartments: skin excess, SC, epidermis + dermis and receptor fluid.

Results
Under the present experimental conditions, for the dye formulation, a mean total recovery of 84.3% has been obtained.
For the aqueous solution sample, a total recovery of 101% has been obtained. Most of the hair dye applied on the skin surface was removed with the washing procedure (83.9 % of the applied dose after 30 min and a further 8.75 % at the end of the exposure period). The content of the test substance detected in the SC was: 3.71 %. A total of 4.2 % of the applied dose (8.4 µg/cm²) is reported to have penetrated into the living skin compartments (epidermis and dermis) and permeated into the receptor fluid during 48 h.

Ref. : 19

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline	:	-
Species/strain	:	*S. typhimurium,* TA98, TA100, TA1535, TA1537, TA 1538
Replicates	:	Triplicate plates, 2 independent tests
Test substance	:	Arianor Straw Yellow dissolved in DMSO
Batch no	:	batch no KS 3131
Purity	:	/
Concentrations	:	5 concentrations – direct plate incorporation assay. with and without metabolic activation Test #1 : 4, 20, 100, 500, 2500 µg/plate Test #2 : 8, 40, 200, 1000, 5000 µg/plate
GLP	:	In compliance

Basic Yellow 57 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.

Results
- 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.
- With 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 Arianor Straw Yellow (batch no KS 3131), is negative in any of the *S. typhimurium* tester strains in the absence or the presence of S9 mix.

Ref. : 11

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

Guideline	:	-
Cells	:	Chinese Hamster V-79 cell line
Replicates	:	2 independent tests
Test substance	:	Arianor Straw Yellow in DMSO solution
Batch no	:	KS 1823
Purity	:	63.5 % dye content
Concentrations	:	Test #1 : 5 concentrations with and without metabolic activation, 30, 100, 200, 300 and 1000 µg/ml Test #2 without S9 : 4 concentrations : 30, 100, 300 and 1000 µg/ml

with S9 : 5 concentrations : 20, 100, 250, 500 and 1000 µg/ml
GLP : In compliance

Arianor Straw Yellow (batch KS1823) has been investigated for gene mutation at the HGPRT locus in V-79 Chinese hamster cell line. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system.
Exponentially growing suspension cultures of V-79 cells were treated with the test agent for : 3 hours with S9 mix and during 24 hours without S9 in the culture medium
The concentration range 150, 300, 750 and 1000µg/ml was selected on the basis of a preliminary toxicity study. Negative and positive controls were in accordance with the OECD guideline (Ethyl methanesulphonate and 9, 10-dimethyl-1,2-benzanthracene).

Results
pH and precipitate
Precipitate occurred at 200 µg/ml. pH measurement of post-treatment medium was not performed.

Cytotoxicity
Test #1 without S9 mix : a decreased cloning efficiency at day 2 was reported from concentration of 30 µg/ml without S9 mix.
Test #1 with S9 mix : no decrease in the cloning efficiency at day 2 was observed with S9 mix.

Test #2 without S9 mix : No substantial decrease in the cloning efficiency at day 2 was reported from concentration of 30 µg/ml without S9mix.
Test #2 with S9 mix : no substantial decrease in the cloning efficiency at day 2 was observed with S9 mix.

Mutant frequency
Test #1 Without S9 mix : A sporadic increase in mutant frequency was observed over the concurrent solvent controls at 1000 µg/ml. However, no dose dependency was noted.

Test #1 With S9 mix : A decrease in mutant frequency was observed over the concurrent solvent controls at 200 µg/ml and upwards, concentration for which precipitate was noted.

Test #2 Without S9 mix : A sporadic increase in mutant frequency was observed over the concurrent solvent controls at 1000 µg/ml.
Test #2 With S9 mix : An large increase in mutant frequency was observed over the concurrent solvent controls at 100 µg/ml and 200 µg/ml; for the two upper concentrations for which precipitate was noted, a decrease in the mutant frequency was observed.

Conclusions
From the results generated in 2 experiments it may be concluded Arianor Straw Yellow (batch KS1823) shows some equivocal positive results. However, the study seems unsuitable for an accurate evaluation because of the possible methodological confounding (purity, different dates for the performance of the studies – 9 May, 10 June and 12 August).

Ref. : 12

***In vitro* mammalian chromosomal aberration test**

Guideline	:	OECD 473
Species/strain	:	Chinese Hamster V79 Cells
Replicates	:	Duplicate cultures
Test substance	:	Arianor Straw Yellow in deionized water
Batch no	:	RS 68216101
Purity	:	> 99.2 area-%, HPLC
Concentrations	:	200-1200 µg/ml with and without metabolic activation.
GLP	:	In compliance

Basic Yellow 57 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 Arianor Straw Yellow, concentrations between 29.7 to 3800 µg/ml (that correspond to approximately 10 mM) have been selected.

Exposure period	Recovery	Preparation interval	Doses µg/ml		
Exp # 1 without S9 mix					
4 hours	14 hours	18 hours	200	400	1200 *
Exp # 2 without S9 mix					
18 hours	none	18 hours	200	400	1000 *
28 hours	none	28 hours		600	
Exp # 1 with S9 mix					
4 hours	14 hours	18 hours	200	400	800 *
Exp # 2 with S9 mix					
4 hours	14 hours	18 hours	200	400	800 *
4 hours	24 hours	28 hours	100	200	800 *

* : precipitation occurred

Results
pH and Osmolarity
In the range finder study, precipitation occurred at 950 µg/ml
At the top concentration, no influence of the pH or osmolarity was noted (solvent control pH: 7.3; 289 mOsm top dose of 3800 µg/ml pH : 7.3; 302 mOsm).

Toxicity
Toxic effects as evidenced by a reduction in cell numbers was observed in the absence or in the presence of S9 mix. The top doses were chosen on the basis of the strong mitotic index reduction and the poor quality of metaphases, the latter factor being relevant for scoring errors

Structural chromosome aberrations

Experiment # 1 and # 2 With or Without activation system.
In both independent experiments, no statistically and/or biologically significant relevant increase in the number of aberrant cells were observed as compared to the corresponding solvent control at any dose and treatment time in the presence or absence of activation system. both independent

assays. It should be noticed that, while not statistically significant, there is a trend for a dose-response relationship in Experiment # 1, with activation (4 hours exposure). The frequency of cells displaying aberrations were as follow :

- 200 µg/ml 0/200
- 400 µg/ml 0.5/200
- 800 µg/ml 1.5/200

However, such frequencies are thought not to be biologically relevant because they fall within the historical control value of the laboratory.

In both experiment , positive control showed distinct increases in the number of aberrant cells and type of aberrations.

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.

Conclusions
The assay is acceptable for evaluation. Arianor Straw Yellow (Batch No RS 68216101 - purity > 99.2 %) is considered negative for clastogenic activity in chinese hamster V79 cell line in the absence or in the presence of activation under the conditions of these tests.

Ref. : 18

Guideline	:	OECD (482)
Species/strain	:	Rats hepatocytes
Replicates	:	2 independent tests
Test substance	:	Arianor Straw Yellow in DMSO solution
Batch no	:	KS 1823
Purity	:	63.5 % dye content
Concentrations	:	Test #1 : 5 concentrations : 0.10, 0.33, 1.0, 3.33 and 10.0 mg/ml Test #2 : 5 concentrations : 0.03, 0.10, 0.33, 1.0and , 3.33 mg/ml
GLP	:	In compliance
Exposure time	:	3 h

Results
Basic Yellow 57 has been investigated for induction of unscheduled DNA synthesis in rats hepatocytes incubated with the test material and ^{3}H-thymidine for three hours. The nuclear DNA was isolated and the incorporation of ^{3}H-thymidine was determined by liquid scintillation counting.
No increase (expressed as dpm/µg DNA) in UDS was noted in both experiments. The reduction in the incorporation of radioactivity that was noted at concentrations of 3.33 mg/ml and 10.00 mg/ml was interpreted as an indication of toxicity.
However, the methodology adopted (scintillation counting) is the less sensitive to be used in this type of test, according to the scientific literature. Moreover, there is an large inter- and intra-

variability in the results (dpm/µg DNA) that gives low weight to this test. In addition, the results are poorly explained in term of biological significance.

Conclusions
This study is unsuitable for an accurate evaluation.

Ref. : 13

2.8.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline	:	OECD 474
Species	:	CFW 1 mouse
Group sizes	:	5 male and 5 female
Material	:	Arianor Straw Yellow dissolved in NaCl solution at 0.9%
Batch no	:	not stated
Dose levels	:	0 and 1000 mg/kg bw in a volume of 20 ml/kg
Administration	:	intragastric gavage
Sacrifice times	:	24, 48 and 72 hours after dosing.
GLP	:	In compliance

Basic Yellow 57 has been investigated for induction of micronuclei in the bone marrow cells of male or female mice. Dose levels were determined by a preliminary range finding study in which observable toxic effects were seen. The substance was administered by a single intragastric gavage and the groups of animals sacrificed 24, 48 and 72 hours after administration. Negative and positive controls were in accordance with the OECD guideline.

Test doses
Arianor Straw Yellow 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 hours 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
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
No significant reduction in the PCE/NCE ratio was observed in any of the dosage groups of mice treated with Arianor Straw Yellow.

Conclusions
Under the conditions of the test it can be concluded that with Arianor Straw Yellow (Batch not stated)at doses at which no significant variation in the PCE/NCE ratio was observed, does not induce statistically significant increase in the frequency of PCE. The negative and positive controls gave the expected results. Therefore, with Arianor Straw Yellow (Batch not stated) is considered not clastogenic and/or aneugenic in this mouse bone marrow micronucleus test.

Ref. : 14

2.11. Safety evaluation

NOT APPLICABLE

2.12. Conclusions

Basic Yellow 57 is not a single compound, but a mixture of a dye with other chemicals. Consequently the chemical name presented for Basic Yellow 57 is not correct. All tests except "percutaneous absorption study" and "In vitro mammalian chromosomal aberration test" reported in Submission II are performed using the dye preparation containing approximately 63.5% 5-Hydroxy-3-methyl-1-phenyl-4-(3'-trimethylammoniophenylazo)-pyrazol, chloride.

The UV spectrum of Basic Yellow 57 shows significant absorbance at 246nm and 383 nm, with λ_{max} at 383 nm. The chromatographic purity using HPLC has been described only by UV detection at 246 nm. The HPLC-UV detection 383 nm of the dye should be reported. Basic Yellow 57 is a mixture of the dye with 9.6% sugar and >20% inorganic salts. The chemical specification of the sugar and inorganic salts are required. The information on the content of volatile organic solvents in the dye, density, Log P_{ow}, quantitative data on the solubility and stability of the dye have not been reported.

Basic Yellow 57 has low acute oral toxicity in mice and rats. The LD_{50} was reported to be greater than 2000 mg/kg bw. In one study in rats, the $LD_{50\ was}$ between 1000 and 2000 mg/kg bw.

Basic Yellow 57 was administered for 12 weeks by oral gavage to Wistar rats at 50 mg/kg bw. The dose level of 50 mg/kg bw/day was considered to be on the borderline of toxicity. In another 13 week study, Basic Yellow 57 was administered by oral gavage to Sprague Dawley rats at 20 mg/kg bw. There were no indications of treatment-related effects from clinical, macroscopic or microscopic examinations. The dose of 20 mg/kg body weight/day was considered to be the NOAEL.
Basic Yellow 57 produced no indications of maternal toxicity, embryo-toxicity or teratogenicity under the test conditions employed at the dose of 50 mg/kg bw/day.

Basic Yellow 57 was considered "not irritant" to rabbit skin. It provoked no effects on the cornea or iris in any of the test animals. However, there was a discoloration of the conjunctivae. A mild reaction could have been masked by the discoloration caused by the dye. This test was conducted at a concentration below the intended in-use maximum of 2%.

Percutaneous absorption study, *in vitro* : the study indicated a maximum penetration of 8.7 µg/cm² of Basic Yellow 57 in a aqueous solution.

Basic Yellow 57 was tested in procaryotic cells for gene mutation in several tester strains of S. typhimurium . The results are negative.
Basic Yellow 57 gave some positive results in the *in vitro* Mammalian Cell Gene Mutation Test. However, the assay is considered unsuitable for evaluation to methodological possible confoundings. The *in vitro* mammalian chromosomal aberration test is acceptable for evaluation and give negative results.
Basic Yellow 57 gave negative results in the unscheduled DNA synthesis (UDS) test with mammalian liver cells *in vitro*. However, the study is considered unsuitable for accurate evaluation.
Basic Yellow 57 gave negative results in the Mammalian Erythrocyte Micronucleus Test. However, the study did not demonstrate that bone marrow was reached by the test agent. Moreover, while conform to OECD guideline 474, no raw data on PCE and NCE are presented.

2.13. References

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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 any further consideration, a complete new re-submission is required on the substance(s) to which the consumer is presently exposed.

4. Other considerations

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5. Minority opinions

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