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

CONCERNING

Basic Yellow 87

adopted by the SCCNFP during the 25th plenary meeting
of 20 October 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 87 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

2.1. General

2.1.1. Primary name

Basic Yellow 87 (INCI)

2.1.2. Synonyms

Chemical name : 1-methyl-4-((methylphenylhydrazono)methyl)-pyridinium, methylsulfate
CAS name : pyridinium, 1-methyl-4-((methylphenylhydrazono)methyl) methylsulfate
Synonyms : /

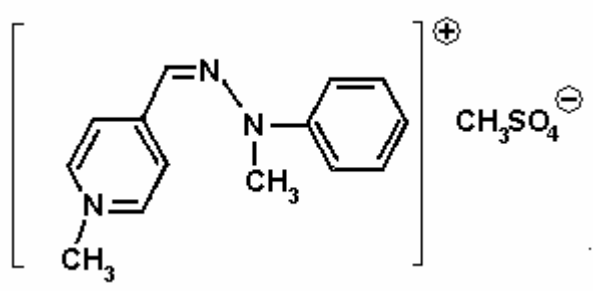
2.1.3. Trade names and abbreviations

Trade name(s) : Vibracolor Citrus Gelb (Vibracolor Citrus Yellow);
MIP 2982; MIT Gelb 2982
COLIPA n° : /

2.1.4. CAS n° / EINECS n°

CAS n° : 68259-00-7
EINECS n° : 269-503-2

1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{15}H_{19}N_3O_4S$ (methosulfate)
Mol weight : 337.4 g/mol (methosulfate)

2.1.7. Purity, composition and substance codes

Purity		
Titre as determined by HPLC	:	88.6 - 92.6 %
Water content	:	≤ 0.5 %
Heavy metals	:	/
Potential impurities	:	≤ 0.1 % coloured by-product
Salts of formulation or counter ions	:	≤ 1.7 % Sodium chloride ≤ 7.8 % Methyl sulfate ≤ 0.9 % sulfate
Reagents and intermediate reaction products	:	/
Solvent residues	:	≤ 0.1 % isopropanol

2.1.8. Physical properties

Appearance	:	Yellow solid
Melting point	:	140° C (135-164 °C), not melting, decomposition at higher temperatures
Boiling point	:	/
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{ow}	:	– 1.69 (OECD n° 107)

2.1.9. Solubility

water : 40 g/l at 20 °C

General comments on analytical and physico-chemical characterisation

* A discrepancy of the salt content in the samples has been noted. During the manufacturing process, the dyestuff is salted out with NaCl. Vacuum drying and recrystallisation will produce high purity dye with less salt content.
* No experimental data is provided on stability of the test material.

2.2. Function and uses

Basic Yellow 87 is used at 0.2 % in semi-permanent (35 ml) and at a final concentration of 0.1 % (100 ml) in oxidative hair dye formulations, after mixing with the oxidative agent.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Guideline	:	/
Species/strain	:	CD rat
Group size	:	2 males + 2 females at 500, 1000, 1500 mg/kg bw, 5 male + 5 female at 2000 mg/kg bw
Test substance	:	MIP 2982 in water
Batch no	:	028400A8AA
Dose	:	500, 1000, 1500 and 2000 mg/kg bw by gavage
Observ. Period	:	14 days
GLP	:	in compliance

The acute oral toxicity was determined in a dose-limit test protocol using 22 animals.
With the exception of enlarged heart (1500 mg/kg, 1 male) and dark areas or dark-red lobes on the lung (500 mg/kg, 1 male, 1 female) no visible lesions were noted in the animals that survived to study termination. The study results indicate a median lethal dose between 500 and 1000 mg/kg bw in female and >1500 mg/kg bw in males, respectively

Ref : 1

2.3.2. Acute dermal toxicity

Guideline	:	OECD 402 (1998)
Species/strain	:	Crl: CD (SD) IGS BR rats, Charles River Laboratories
Group size	:	5 male, 5 female CD rats
Test substance	:	MIP 2982
Batch no.	:	028400A8AA
Purity	:	87.7 %
Dose	:	A single dose of 2000 mg/kg applied occlusively for 24 hours to an area of approximately 10% of the total surface area.
GLP	:	In compliance

The test article was moistened with water and applied as a uniform layer to the skin and covered with an occlusive dressing and bandage for 24 hours. Residual test article was removed with water and soft paper towel. All animals were observed for systemic effects and dermal toxicity on days 3, 7, 10 and 14.

Results
There were no signs of dermal toxicity.

Ref.: 2

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

Guideline	:	OECD 407 (1995) and 96/54/EEC
Species/strain	:	HanIbm: WIST rat
Group size	:	5 males + 5 females, 10 male + 10 female controls and high dose
Test substance	:	MIP 2982 in feed
Batch number	:	CGF-F016737/0016
Purity	:	> 92 %
Dose levels	:	0, 10, 50 and 250 mg/kg bw target doses
Exposure period	:	28 days, ad libitum
GLP	:	in compliance

60 rats were used in the study. The test substance was administered in feed admixture while the controls received the normal diet. Dosages achieved were 8, 38.8, and 174 mg/kg bw/day in males and 8.2, 40, and 184 mg/kg bw/day in females, respectively, as calculated from food consumption and body weight data. All animals were observed daily for clinical signs and mortality. Body weights, food and water consumption were recorded in weekly intervals. A functional observational battery was performed during week 4 on 5 rats per group and sex; behavioral abnormalities were screened. Urine was collected after 4 and 6 weeks. All animals were sacrificed at the end of the study. Organ weights were recorded, macroscopy was performed on all animals. Samples of major organs from all control and high dose animals as well as liver and thyroid glands and all gross lesions from all animals were processed as hematoxylin-eosin slides and examined by light microscopy.

Results
Yellow discoloration of the faeces was noted in all rats of the high dose group and deep yellow urine discoloration was observed in all animals receiving the substance. There were no effects on haematology, clinical biochemistry and urinalysis that were considered of toxicological significance. The functional observational battery did not reveal abnormal test article related findings. In high dose males the food intake and the mean body weight and body weight gain was slightly lower. A slightly reduced total protein and globulin level and a slightly increased albumin to globulin ratio were recorded in males of the high dose group.

The NOAEL is 174 mg/kg bw/day, the NOEL approximately 39 mg/kg bw/day.

Ref.: 4

2.3.5 Repeated dose dermal toxicity

Guideline	:	OECD 402 (1987)
Species/strain	:	Himalayan spotted guinea pigs
Group size	:	4 males, 4 females
Test substance	:	MIP 2982
Batch number	:	CGF-FO16737/0016
Purity	:	>92%
Dose levels	:	0.1 ml/7 cm^2, 4 concentrations 5%, 3%, 1% and 0.5%
GLP	:	In compliance

Two applications sites of 7 cm^2 were marked on the shaved back of 6 test article treated animals. Two animals were controlled and treated with vehicle. A complete block design was used so each concentration was tested three times on three different animals. 0.1 ml was applied daily on

each test area, which was left open. The treated skin was flushed with water prior to each new application. The animals were shaved regularly and depilated on day 15, one hour prior to final reading. Skin reactions were observed daily and scored by used of a 5 point ranking scale.

Results
No grading scores were recorded from day 2-14 due to slight accumulation of test article on the skin. On test day 15 after depilation no skin reaction was observed.

Ref. : 3

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Subchronic oral toxicity

Guideline	:	OECD 408 (1981) and 96/54/EEC
Species/strain	:	Wistar rat SPF-bred
Group size	:	10 males + 10 females
Test substance	:	MIP 2982 in feed
Batch number	:	CGF-F016737/0016
Purity	:	>92 %
Dose levels	:	0, 10, 50, and 250 mg/kg bw/day target doses
Exposure period	:	13 weeks
GLP	:	in compliance

80 rats were used in the study. The test substance was administered in feed admixture while the controls received the normal diet. Dosages achieved were 9.7, 48.5, and 245.2 mg/kg bw/day in males and 10.1, 48.9, and 245.0 mg/kg bw/day in females, respectively, as calculated from food consumption and body weight data. All animals were observed daily for clinical signs and mortality. Ophthalmoscopic examination was performed at pretest and at week 13 in the control and high-dose animals. Body weights, food and water consumption were recorded in weekly intervals. A functional observational battery was performed during pretest and at week 12 on all rats. At 13 weeks, blood samples for haematology and clinical biochemitry as well as urine samples were collected from all animals. All animals were weighed and sacrificed at the end of the study. Organ weights were recorded, macroscopy was performed on all animals. Samples of major organs from all control and high dose animals as well as liver and thyroid glands and all gross lesions from all animals were processed as hematoxylin-eosin slides and examined by light microscopy.

Results
No test article related ophthalmologic findings were noted. No adverse findings were seen in the functional observational battery. In the high and mid-dose group coloured faeces in both sexes were observed, which in the high dose group was accompanied by a deep-yellow urine discoloration. In females the urine pH was increased only in the high dose group, while a decrease in the uric acid level in females was noted in all dose groups. The total bilirubin levels were decreased in females in the mid and high dose group. The following effects were only seen in the high dose group: Reduced food intake and body weight gain (males), increase in Met-Haemoglobin levels in both sexes, white blood cells number decreased (males), increased platelet count (females), changes in creatinine levels, total protein amount, glucose levels, changes in several organ/body weight as well as organ/brain ratios.

The NOAEL is 10 mg/kg bw/day.

Ref.: 5

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)

Guideline	:	OECD 404 (1981)
Species/strain	:	New Zealand albino rabbit
Group size	:	2 male, 1 female
Test substance	:	MIP 2982, moistened with water
Batch no	:	028400A8AA
Purity	:	87.7 %
Dose	:	0.5 g
GLP	:	In compliance

0.5 g of moistened test substance was applied to 6.25 cm^2 of intact skin of 3 rabbits. Semi occlusive bandages were applied and left for 4 hours. Remaining test substance was rinsed off. The skin was examined for skin changes at 0.5 to 1, 24, 48 and 72 hours after patch removal.

Results
No findings of erythema or oedema were noted in any of the animals. The test article was non-irritating to rabbit skin.

Ref. : 6

2.4.2. Irritation (mucous membranes)

Guideline	:	OECD 405 (1987 – with any applicable amendments)
Species/strain	:	New Zealand albino rabbits
Group size	:	1 male, 2 females
Test substance	:	MIP 2982
Batch no.	:	Lot 028400A8AA
Purity	:	87.7 %
Dose	:	0.1 ml, approximately 0.057 g of test article
GLP	:	In compliance

0.1 ml of the test article was applied once to the right eye of the rabbits, without rinsing. The left eye served as control and was untreated. Ocular reactions were recorded at 1, 24, 48, 72 and 96 hours and 7, 14 and 21 days after installation.

Results
There were no changes involving the cornea. There was a minimal circumcorneal injection with the iris reacting to light at 1 hour post installation. These findings were resolved by 24 hours. Redness was seen in all animals from 1 to 72 hours, and in one animal at 7, 14, and 21 days after installation. Chemosis and discharge were noted in all animals up to 48 hours post installation. The maximum mean score was 9.0 at 1 hour post installation.
MIP 2982 was moderately irritating to rabbit eyes under conditions of this study.

Ref. : 7

2.5. Sensitisation

Guinea Pig Maximization Test

Guideline	:	OECD 406 (1992)		
Species/strain	:	Himalayan spotted albino guinea pigs		
Group size	:	15 female animals, 10 test and 5 control		
Test substance	:	MIP 2982		
Batch no.	:	CGF-FO16737/0016		
Purity	:	>92%		
Concentration	:	Intradermal induction	:	1% in water
		Topical induction	:	50% in water
		Challenge	:	50% in water
GLP	:	In compliance		

The concentrations were selected based on pilot studies. Intradermal induction was performed with a 1% dilution of test article in water with and without Freund's Complete Adjuvant. Prior to topical induction the test sites were pretreated with 10% SLS for 24 hours. Control animals were treated with vehicle without MIP2982. Two weeks after topical induction the animals were challenged with occlusive patches, and reactions were evaluated at 24 and 48 hours after removal of patches. Yellow discoloration was noted and all animals were depilated approximately 3 hours prior to reading.

Results
None of the animals showed any reaction. Under the test conditions MIP 2982 was not a sensitiser.

Ref. : 8

2.6. Teratogenicity

Guideline	:	OECD 414 (1981)
Species/strain	:	Wistar rat
Group size	:	22 mated females per group
Test substance	:	MIP 2982 in 4 % CMC in bidistilled water
Batch number	:	CGF-F016737/0016
Purity	:	>92 %
Dose levels	:	0, 20, 60, 180 mg/kg bw by gavage

Treatment period	:	day 6-17 post coitum
GLP	:	in compliance

Groups of 22 mated female rats received orally MIP 2982 by gavage once daily at dose levels 20, 60, and 180 mg/kg bw from day 6 to 17 post coitum. The controls received only the vehicle. Food consumption, body weight, mortality and clinical signs were recorded. Gross necropsy was performed on day 21 and the maternal organs were examined. The uteri were weighed, the foetuses removed, weighed, and examined for sex and gross external abnormalities.

Maternal deaths did not occur and no clinical signs were noted except for yellow faeces and/or urine in the 60 and 180 mg/kg bw groups. At these dose levels reduced food consumption and weight gain were observed. No substance-related changes of reproduction data were noted (number of implantations, resorptions and foetuses, foetal weight, external abnormalities) with the exception of 1 foetus with cleft palate (20 mg/kg bw) and 1 oedematous foetus and a slight increase in foetal weight (180 mg/kg bw). Some observed skeletal abnormalities were not considered to be related to the test substance.

The NOAEL of maternal and foetal toxicity is 60 mg/kg bw.

Ref.: 11

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Percutaneous absorption in vitro

Guideline	:	/
Tissue	:	Human female epidermis, heat-separated
Method	:	Franz diffusion cells (static) 12 cells dosed with test article and 2 with a placebo preparation
Test substance	:	A formulation containing 0.2% MIP 2982
Batch number	:	CGF-FO16737/16
Dose levels	:	90.2-109 mg/cm² (mean 103 ± 1.8 mg/cm²) preparation equals 197 ± 3.3 µ/cm² of MIP2982
GLP	:	In compliance

The diffusion cells were kept in water bath maintaining a skin surface temperature at 32.0 ± 0.1°C. Barrier integrity was checked with penetration of Tritium labelled water. The receptor phase was pH 7.4 phosphate buffered saline/25% ethanol. The formulation was applied to the skin surface in a representative hair dye formulation at a target dose of 100 mg/cm². After 30 min. exposure period the skin surface was rinsed with warm (40°C) water. Permeation of MIP 2982 through the skin was monitored over 48 hours. The cells were then dismantled and a surface wipe, donor chamber rinse, filter paper support, tape strips and the remaining skin samples were analysed for MIP 2982 content and a full mass balance calculated. Samples were analysed for MIP 2982 by HPLC. The detection limit was 2 ng/ml.

Results
The overall recovery of the applied dose was 98 ± 0.1%. Permeation of MIP 2982 through the skin was detected in all, but one of the cells treated with the hair dye formulation. The total percutaneous absorption (material in the remaining skin and the receptor phase) of MIP 2982 from the hair dye formulation after a 30 min. exposure period amounted to 0.082 ± 0.010 % of the applied dose, equal to 0.16 ± 0.031 µg/cm².

Comment
The substance was not tested in the presence of an oxidising agent. The applied dose is higher than the amount recommended by the SCCNFP (20 mg/cm²).

Ref.: 12

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline	:	OECD 471
Species/strain	:	*S. typhimurium,* TA98, TA100, TA1535, TA1537, *E. coli* WP2 uvrA
Replicates	:	Triplicate plates, 2 independent tests
Test substance	:	MIP 2982 in water
Batch no	:	028400A8AA, purity : 87.7 %
Concentrations	:	*S. typhimurium* and *E. coli* Test #1 Without metabolic activation 33.3, 100, 333, 1000, 2000, 3330 µg/plate (6 doses) With metabolic activation (rat liver) 33.3, 100, 333, 1000, 3330, 5000 µg/plate (6 doses) With reductive metabolic activation (hamster liver) 33.3, 100, 333, 1000, 3330, 5000 µg/plate (6 doses) Test #2 Without metabolic activation 33.3, 100, 333, 1000, 2000, 3330 µg/plate (6 doses) With metabolic activation (rat liver) 33.3, 100, 333, 1000, 2000, 3330, 5000 µg/plate (6 doses) With reductive metabolic activation (hamster liver) 33.3, 100, 333, 1000, 2000, 3330, 5000 µg/plate (6 doses)
GLP	:	In compliance

Basic Yellow 87 has been investigated for gene mutation in *Salmonella typhimurium and E. coli* using the preincubation plate incorporation method both with or without S9 mix.
S9 mix from different origin have been used : Standard : Sprague-Dawley rats injected i.p. with Aroclor™ 1254 ; Reductive : uninduced male Golden Syrian hamsters.
Negative and positive controls were in accordance with the OECD guidelines.

Results
Dose range finding assay
Inhibition of growth, as evidenced by a decrease of revertant frequency or thinning of the background lawn was observed in the tester strains

* TA 100 at doses ≥ 667 µg/plate in the presence of S9 and;
  at doses ≥ 100 µg/plate in the absence of S9.
* *E. coli* at doses ≥ 1000 µg/plate in the presence of S9 and;
  at doses ≥ 667 µg/plate in the absence of S9

Test # 1
In the absence of activation, no dose related and biologically relevant increase in revertant numbers was observed, in any tester strains.
In the presence of rat (rat liver S9) activation : No dose related and biologically relevant increase in revertant numbers was observed, in any of the tester strains used (Salmonella or E. coli).
In the presence of Hamster (reductive S9) activation : an increase in revertant numbers was observed for both a frameshift (TA 1537) and a base-pair substitution strain (*E. coli*) at the 1000 µg/plate dose. This increase is observed in the 3 plates set (49 ± 5; TA 1537) and (33 ± 7 ; *E. coli*). Although, this increase is not dose related it should be noted that for the following dose cytotoxicity was observed that could have prevented the expression of increased mutants frequencies. On the other hand, the extent of the increase observed in *E. coli* is in the range of values observed sometimes in control ranges.
For the other strains, no statistically or biologically relevant increase of mutant frequencies have been observed as compared to the controls.
Positive controls showed the expected response.

Test # 2
Mutation test # 2 (confirmatory) Reversion rate
An additional dose close to the one that gave a positive finding was set up.
In the absence of activation : No dose related and biologically relevant increase in revertant numbers was observed, in any of the tester strains.
In the presence of rat activation : No dose related and biologically relevant increase in revertant numbers was observed, in any of the tester strains.
In the presence of Hamster (reductive S9) activation : No statistical significant increase in revertant numbers was observed for any tester strains (TA 1537; *E. coli*) and, according to the positivity criteria, no significant positive findings were observed neither at 1000 µg/plate not for the additional dose of 2000 µg/plate. Moreover, the increases are not dose related and therefore considered as devoid of biological significance. In addition, attention should be paid to the fact that such frequencies are observed in the presence of reductive S9 mix and might be due to the presence of aromatic metabolites.

Conclusions
Based on the reversion rate, and under the conditions of the 2 assays performed in the presence of "normal" and "reductive" S9 mix, it is concluded that the test agent MIP 2982 dissolved in water has no mutagenic potential in the different bacterial tester strains used.

Ref. : 13

In Vitro Mammalian Cell Gene Mutation Test

Guideline	:	OECD 476
Cells	:	Chinese Hamster V-79 cell line (mutation at the HPRT locus)
Replicates	:	2 independent tests
Test substance	:	MIP 2982
Batch no	:	CGF-F016737/0013
Purity	:	88.6 % (HPLC)
Concentrations	:	Test #1 Without metabolic activation : 1.0*, 3.0, 10.0, 30.0, 100.0, and 300.0** µg/ml (6 doses)

With metabolic activation :
1.0*, 3.0, 10.0,* 30.0, 100.0, and 300.0 µg/ml (6 doses)
**: culture not evaluated; ** no evaluation due to strong toxicity.*
Test #2
Without metabolic activation :
3.0, 10.0*, 30.0, 50.0*, 100.0, and 200.0 µg/ml (6 doses)
With metabolic activation :
30.0, 50.0, 100.0, 300.0, 450,0 and 600.0** µg/ml (6 doses)

GLP : In compliance

MIP 2982 has been investigated for gene mutation at the HGPRT locus in V79 Chinese hamster cell line in the presence or absence of activation system. No visible precipitate occurred. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system.

Results

Test # 1
Mutant frequencies in the absence or presence of activation.
All doses tested could have been defined as positive according to the positivity criteria. However, the negative control value of 1.1 mutant per 10^6 cells is extremely low (as compared to historical published values) and contributes to the statistical significance observed when compared with exposed cells. Therefore the increases observed are considered devoid of biological significance. (control 1.1 per million cells/ - S9 ; + S9 ctrl 8.3 per million cells). In addition, no dose-response trend was noted.

Test # 2
Mutant frequencies in the absence or presence of activation.
No statistically or biologically significant increase in mutant frequency was observed over the concurrent solvent controls for any doses. No indication for a positive dose-response trend was noted. (ctrl 13.6 per million cells/ - S9 ; + S9 ctrl 10.2 per million cells/ + S9)

Conclusions
No biologically relevant significant increase in mutant frequency was observed over the concurrent solvent controls after treatment with MIP 2982 in either test in the presence or absence of activation.
Therefore, the test substance MIP 2982 does not demonstrate mutagenic potential on the HPRT gene of V79 cells.

Remark
It should be noted that the test agent expresses a clear cytotoxic effect. In addition, for a better understanding of some positive *in vitro* results observed so far in the Ames test, it should have been interesting to compare the mutagenic potential of metabolites at the gene level in mammalian cells under similar conditions of activation systems (Hamster reductive S9 activation) that gave some sporadic positive results in 2 different bacterial tester strains (TA 1537 & *E. Coli*).
No conclusion can be made at present.

Ref. : 15

***In Vitro* Mammalian Chromosomal Aberration Test**

Guideline	:	OECD 473
Species/strain	:	Human lymphocytes (non pooled cultured blood samples)
Replicates	:	Duplicate cultures, 2 independent experiments
Test substance	:	MIP 2982 in water
Batch no	:	028400A8AA
Purity	:	90.5 % (HPLC)
Concentrations	:	Test #1 without S9 mix 3 h treatment – 22 h harvest : 33.9, 48.4, 69.1, 98.7 µg/ml with S9 mix 3 h treatment – 22 h harvest : 69.1, 98.7, 202.0, 288.0 µg/ml Test # 2 without S9 mix 22 h treatment – 22 h harvest : 3.55, 7.10, 14.2 µg/ml with S9 mix 3 h treatment – 22 h harvest : 57.8, 92.8, 155.0, 206.0 µg/ml
GLP	:	In compliance

MIP 2982 in water has been investigated for induction of chromosomal aberrations in human non pooled lymphocytes. 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.

Results
pH & Osmolarity

* At the maximum dose tested of 5000 µg/ml, the pH was not significantly changed (pH =7.5)
* Osmolarity measurements of post treatment medium was performed for the top dose of the dose range finding (3500 µg/ml) ; it has been found to exceed slightly the maximum value accepted 10 mM (10 mM = 3337 µg/ml). However, this can be considered as devoid of relevance for the final assay.

Chromosome aberrations

* For the dose of 98.7 µg/ml, an excessive cytotoxicity has been observed that prevented scoring of metaphases (MI = 0.1 %). However, this toxicity occurred in one flask only, therefore, the required number of cells (n = 200) could be scored on slides from the remaining flask. This deviation does not alter neither the validity of the test, nor the results.
* No statistically or biologically significant increase in the number of aberrant cells was observed as compared to the corresponding solvent control.

Polyploidy
No significant increase of aneuploidy and/or endoreduplicated cells was noted.

Conclusions
MIP 2982 could be considered negative for clastogenic and/or aneugenic activity in human lymphocytes in the presence or the absence of rat activation system under the conditions of the test. Because of methodological inadequacies, a conclusion cannot be made.

Ref. : 14

2.8.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline	:	OECD 474 (1983)
Species	:	NMRI mice
Group sizes	:	6 male and 6 female
Material	:	MIP 2982 in deionized water
Batch no	:	CGF-F016737/0013
Purity	:	88.6 % (HPLC)
Dose levels	:	Maximum Tolerated Dose (MTD) prelimiminary dose-range finding assays were conducted. According to clinical signs and toxic reactions of the mice, the top dose has been chosen to be 125 mg/kg B.W. MIP 2982 was administered by 1 single oral dose of : * 12.5, 40.0 and 125 mg/kg bw for the 24 h sacrifice time * 125 mg/kg B.W. for the 48 h sacrifice time.
GLP	:	In compliance

MIP 2982 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 at doses of 1000 and 2000 mg/bw. 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.
Number of cells scored : a total of at least 2000 erythrocytes were examined from each animal ; the incidence of micronucleated erythrocytes and the ratio of polychromatic erythrocytes to normochromatic erythrocytes were calculated.

Results

* NCE : the mean number of NCE (mature differentiated cells) was not significantly increased after treatment as compared to controls; this reflects the lack of cytotoxicity of the test agent.
* PCE 24 h sampling time : no statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic erythrocytes over the concurrent vehicle control values were observed for any dose levels.
* PCE 48 h sampling time : no statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic erythrocytes over the concurrent vehicle control values were observed.

Conclusions
Under the conditions of the test it can be concluded that MIP 2982 at doses at which some signs of clinical toxicity were recorded, does not induce statistically significant increase in the frequency of μ PCE.
There is no evidence that the chemical has reached the target cell.

Ref. : 17

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

Guideline	:	OECD draft guideline 486
Species/strain	:	Wistar rat, HanIbm: WIST (SPF) strain
Group size	:	4 male rats
Test substance	:	MIP 2982
Batch No	:	CGF-F016737/0013
Purity	:	88.6 % (HPLC)
Dose levels	:	Maximum Tolerated Dose (MTD) : 3 prelimiminary dose-range finding assays were conducted. The top dose of MIP 2982 was chosen on the basis of clinical signs and toxic reactions of the treated rats; the top dose has been chosen to be 500 mg/kg bw A single oral dose was given to a group of male rats at dose levels of 250 and 500 mg/kg. Two sampling times were selected : 2 h & 16 h post-treatment.
Exposure time	:	2 h and 16 hours: all dose groups
GLP	:	In compliance

MIP 2982 has been investigated for induction of unscheduled DNA synthesis in rats hepatocytes at 2 doses 250 and 500 mg/kg bw.
Positive controls are in accordance with OECD guideline and UDS analysed by autoradiography.
3 males were used per dose/time sampling

Results
* The viability of the hepatocytes was not substantially affected by the treatments
* Treatment with MIP 2982 at doses of 250 & 500 mg/kg yielded group mean NNG values less than 0 for both experiment time and caused no significant increases, as compared to control, in the mean nuclear grain counts.
* The percentage of cells in repair did not significantly differ from the control group.

Conclusions
Data indicate that single oral gavage treatment of male rats dosed once with 250 & 500 mg/kg of MIP 2982 did not induced increased unscheduled DNA synthesis in hepatocytes isolated approximately 2 or 16 hours after dosing.
Under the experimental conditions, it is concluded that MIP 2982 did not display DNA repair activities detectable by this assay.

Ref. : 16

2.9. Carcinogenicity

No data

2.10. Special investigations

Phototoxicity

Guideline	:	OECD. Draft proposal (1995)
Species/strain	:	Himalayan spotted albino guinea pigs
Group size	:	15 female animals, 10 tests and 5 control animals
Test substance	:	MIP 2982

Batch no	:	CGF-FO16737/0016
Dose levels	:	0.025 ml/cm^2 of test article dilution in concentrations 50%, 25%, 15% and 10% in water
UV source	:	Philips Actinic "TLD" lamps (36 w/08) with spectrum 320-400 nm, irradiation dose 20 J/cm² UVA
GLP	:	In compliance

All animals were pretreated with 2% DMSO in ethanol to enhance skin penetration of test substance. The test preparations were made immediately prior to dosing and applied topically and openly to skin areas of 2 cm² on both flanks of the guinea pigs. Thirty minutes after application of the test article, the left flank of the animals was exposed to 20 J/cm² UVA irradiation. The right flank remained unexposed to light and served as a reference site. Control animals were exposed to UVA and vehicle. Skin reactions were evaluated according to a ranking scale at 24, 48 and 72 hours after application.

Results
Phototoxic reactions were observed after test article administration in 6 (at 50%) and 3 (at 25%) out of 10 animals at the 24 hour reading. The positive reactions observed after 24 hours on the non-radiated skin site of one (at 50%) and two (at 25%) out of 10 animals were judged to be incidental and animal specific and not related to test article treatment. No reaction was seen at 48 and 72 hours.

Ref.: 9

Photoallergenicity

Guideline	:	OECD. Draft proposal (1995)
Species/strain	:	Himalayan spotted albino guinea pigs
Group size	:	20 tests and 10 control animals
Test substance	:	MIP 2982
Batch no	:	CGF-FO16737/0016
Dose levels	:	0.1 ml/8 cm² of test article 50% in water
UV source	:	UVA, Philips Actinic TLD lamp (36 w/08) Induction: UVB, Philips Sunlamp TL 20 w/12 Challenge: UVA, 320-400 nm, 10 J/cm², Philips Actinic TLD lamps. The target distance was adjusted to a radiation energy of approximately 2.0 mW/cm²
GLP	:	In compliance

For the induction MIP 2982 was applied epicutaneously to an area of 8 cm² in the nuchal region (marked previously with 4 intradermal injections of Freund's Complete Adjuvant/physiological saline). The test sites were then exposed to 1.8 J/cm² UVB and 10 J/cm² UVA. This procedure was repeated 4 times within 2 weeks. Controls were treated with vehicle alone during induction. Three weeks after beginning of induction a challenge was carried out by treating the guinea pigs epicutaneously on both flanks with the test article at the concentration of 50%, 25%, 15% and 10% in water. Treated sites were then irradiated with UVA 10 J/cm² or left unirradiated, skin reactions were evaluated according to a ranking scale at 24, 48 and 72 hours after challenge exposure.

Results
No reactions were observed on both the irradiated and non-irradiated flanks of test and control animals.

Ref.: 10

2.11. Safety evaluation

Not applicable

2.12. Conclusions

No experimental data is provided on stability of the test material.

A repeated dose oral and dermal toxicity study were performed. The NOAEL in a subchronic toxicity study was set at 10 mg/kg bw/day. The NOAEL for maternal and foetal toxicity was set at 60 mg/kg bw.

Basic Yellow 87 was not irritating to rabbit skin. It was moderately irritating to rabbit eye under the test conditions. In a guinea pig maximization test Basic Yellow 87 was not a sensitizer under the test conditions.

The total percutaneous absorption amounted to 0.16 +/- 0.031 µg/cm². The percutaneous absorption of Basic Yellow 87 has not been tested in the presence of an oxidising agent.

Basic Yellow 87 showed phototoxic potential in the guinea pigs at concentration of 50% and 25% in water. No reaction was observed at the concentration of 15%.

Basic Yellow 87 has been tested in prokaryotic and mammalian cells for gene mutation, and in mammalian cells for chromosomal aberration in vitro. Two *in vivo* tests have been performed (bone marrow micronucleus and UDS tests).
The *in vitro* test for gene mutation in prokaryotes has been found negative also in the presence of a reducing metabolic activation system.
The *in vitro* test for gene mutation in mammalian cells showed that the test agent is non-mutagenic in the absence of activation system and under normal activation conditions.
The *in vitro* test for clastogenicity in human lymphocytes is negative, in the presence of a normal activation system.
The *in vivo* micronucleus test in mice is inadequate. There is no evidence that the test compound reached the target cells.
The *in vivo/in vitro* UDS on rat hepatocytes is negative for the treatment of 2 and 16 hours.
No conclusions can be made at present, due to methodological inadequacies in the in vitro mammalian cell mutation test.

2.13. References

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3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the information submitted is insufficient to allow an adequate risk assessment to be carried out. Accordingly, the SCCNFP considers that it is not possible to assess the safe use of the substance.

Before any further consideration, the following information is required :

* information on the stability of the test material in the test solutions and in the hair dye formulations;
* a percutaneous absorption study in accordance with the Notes of Guidance;
* data on the genotoxicity/mutagenicity following the relevant SCCNFP-opinions and in accordance with the Notes of Guidance.

4. Other considerations

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5. Minority opinions

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