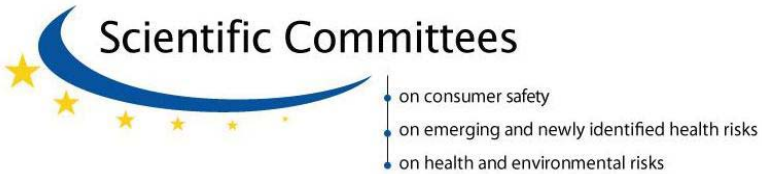
Scientific Committee on Consumer Safety

SCCS

OPINION ON

Basic Yellow 87

COLIPA n° B117



The SCCS adopted this opinion at its 13th plenary meeting

of 13-14 December 2011

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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TABLE OF CONTENTS

1. BACKGROUND

Based on submission I the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) adopted at its 25th plenary meeting on 20 October 2003 its opinion (SCCNFP/0730/03) on Basic Yellow 87 with the chemical name 1-methyl-4-((methylphenylhydrazono)methyl)pyridinium methylsulfate. According to the previous opinion further information were needed on:

- The stability of the test material in the test solution 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

Submission II was submitted in July 2005. According to the conclusion of this submission Basic Yellow 87 is a hair dyeing ingredient intended be used in oxidative hair dyes at 2% concentration (which after mixing in a 1:1 ratio with hydrogen peroxide just prior to use, corresponds to a concentration of 1% on head) and it is also used at 1.0% in non-oxidative hair dye formulations.
The applied hair dye formulations are rinsed off after about 30 minutes and the normal frequency of application being about once per month.

Submission II presents updated scientific data on the above mentioned substance in line with the second step of the strategy for the evaluation of hair dyes (http://europa.eu.int/comm/enterprise/cosmetics/doc/hairdyestrategyinternet.pdf) within the framework of the Cosmetics Directive 76/768/EEC.

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Safety (SCCS) consider Basic Yellow 87 to be safe for use in non-oxidative and oxidative hair dye formulations up to a concentration of 1.0% on-head taken into account the scientific data provided?*

2. *Does the SCCS recommend any restrictions with regard to the use of Basic Yellow 87 in non-oxidative and oxidative hair dye formulations?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Basic Yellow 87 (INCI)

3.1.1.2. Chemical names

1-methyl-4-((methylphenylhydrazono)methyl)-pyridinium, methylsulfate
pyridinium, 1-methyl-4-((methylphenylhydrazono)methyl) methylsulfate

3.1.1.3 Trade names and abbreviations

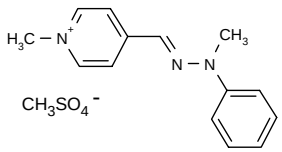
MIP YELLOW 2982
MIP 2982
Yellow (MIP 2982)
Yellow 2982
VIBRACOLOR® Citrus Yellow

COLIPA B117

3.1.1.4 CAS /EC number

CAS: 68259-00-7
EC: 269-503-2

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

Formula: $C_{15}H_{19}N_3O_4S$ (methosulfate)

3.1.2 Physical form

Yellow solid

3.1.3 Molecular weight

Molecular weight: 337.4 g/mol (methosulfate)

3.1.4 Purity, composition and substance codes

Purity and impurities for batch Lot 04143CL2

Identity of batch Lot 04143CL2 was confirmed by 1H-NMR, 13C-NMR, IR, UV at 415 nm
Purity was determined by HPLC-UV at 430 nm after chromatographic separation to be 61.1%.
Methyl sulphate content was 35.7%, determined by IC
Loss on drying: 0.6%
Water: 0.3%

Elemental analysis: 49.7% C, 11.4% N, 1.3% Na

Metal impurities:

Antimony:	< 2 mg/kg
Arsenical:	< 2 mg/kg
Barium:	< 2 mg/kg
Bismuth:	< 2 mg/kg
Cadmium:	< 1 mg/kg
Chromium:	< 2 mg/kg
Copper:	< 2 mg/kg
Lead:	< 2 mg/kg
Manganese:	< 2 mg/kg
Mercury:	< 1 mg/kg
Nickel:	< 2 mg/kg
Silver:	< 2 mg/kg
Tin:	< 2 mg/kg

Comments
In the original paper, there is no information on the methods which are used for the determination of these metals.

Ref.: A

Batch Lot 028400A8AA

Identity was confirmed by IR and UV-Vis
Purity was determined by HPLC as 90.5% and by UV-Vis as 87.7%.

Content of

water	0.2%
2-propanol	0.1%
chloride	<0.1%
sodium	1.6%
sulphate	0.9%
methylsulfate	7.5%

Comments
The methods for the determination of these impurities have not been given in the original data.

Ref.: Analysis Lot 028400A8AA (B117), Sub1

Batch CGF-F016737.0016 (comment: no further details available)

Purity was determined by HPLC to be 92.2% and by UV-Vis to be 92.9%

impurities by HPLC: < 0.1%
content of water: 0.2% by Karl Fischer
content of sodium methyl sulphate: 7.5 % by ion chromatography

Comments
According to the applicants, a reference material has been used for the quantification by HPLC.

Ref.: Analysis – Lot 0016(B117)

Batch CGF-F016737/0013 (comment: no further details available)

Identity was confirmed by IR and UV-Vis spectroscopy.
Purity was determined by HPLC as 88.6%

Content of

water	0.5%
sodium	1.1%
chloride	< 0.1%
methylsulfate	7.4%
sulphate	<0.1%
2-propanol	<0.1%

Ref.: Lot 0013(B117)

Comments
According to the applicants, a reference material has been used for the quantification by HPLC. There is no information in the original paper on how the impurities have been determined.

3.1.5 Impurities / accompanying contaminants

See above

3.1.6 Solubility

Taken from opinion 2003

Water: 40 g/l at 20 °C

3.1.7 Partition coefficient (Log P_{ow})

Log $P_{o/w}$: - 1.69 (OECD 107, flash-shaking method)

Ref.: B

3.1.8 Additional physicochemical specifications

Melting point: 140 °C (135-164 °C), decomposition at higher temperatures
Boiling point:
Flash point:
Vapour pressure:
Density:
Viscosity:
pKa:
Refractive index:
UV/Visible spectrum:

3.1.9. Stability

Stability and homogeneity of the test substance (CGF-F016737/0016) have been tested during the investigations of repeated toxicity. HPLC determinations showed that the test substance was stable and homogeneous in the diet pellets (for rats) within 21 days. The results varied between -3 and +3% of the average concentration.

Ref.: 4, 5

Basic Yellow 87(CGF-F016737/0016) in a solution of 4% CMC in water was stable for 4 hours at room temperature. The individual concentrations varied in the range from -7 to +3% of the mean concentration.

Ref.: 11

The stability of Basic Yellow 87 has been studied under oxidative conditions. Using an HPLC procedure with diode array detector the content of Basic Yellow 87 had been determined before, directly and 30 minutes after applying of peroxide based developer. This, according to the applicants, resembles real use condition of this hair dye. No change in the concentration of Basic Yellow 87 could be observed.

No data in respect of stability of Basic Yellow 87 in hair dye formulations have been supplied.

General Comments on Physico-chemical characterisation

- For the identification of all (4) batches IR spectroscopy and UV/VIS- spectroscopy (190-900 nm) have been used. Batch Lot 04143CL2 additionally was identified by NMR spectroscopy.
- For the quantification of UV/VIS absorbing by-products in all batches, HPLC (415 nm) was used. A reference material served for quantification. That means that the results are quantitative.
- For the quantification of further impurities the following methods have been used:
  In the case of batch Lot 04143CL2 the original paper shows that water content was determined by Karl-Fisher titration. Sulphate, methyl sulphate and chloride were quantified by ion chromatography. 2-Propanol was determined by GC. The content of sodium has been determined by elemental analysis.
  For the other 3 batches the methods for the determination of these impurities have not been supplied in the original papers.
- Stability and homogeneity of the test substance in the formulations used for toxicity testing, have been examined in most studies on percutaneous absorption, repeated and reproductive toxicity, mutagenicity and toxicokinetics.
- No data in respect of stability of the hair dye formulations containing MIP YELLOW 2982 have been supplied.
- All batches analysed contain (mono)methyl sulphate (7.4 to 35.7%), which is used as an anion to the dye. No specific toxicity data for monomethyl sulphate were provided. However, the toxicity testing was performed in the presence of this anion.

Annex I shows detailed information about identity, purity, impurities, stability and homogeneity of the formulations used for the testing of the toxicity (…) of Basic Yellow 87.

3.2. Function and uses

Basic Yellow 87 (MIP YELLOW 2982) is used at concentrations up to 2% in oxidative hair dyeing systems (which after mixing in a 1:1 ratio with hydrogen peroxide just prior to use, corresponds to a concentration of 1% upon application) and up to 1% in non-oxidative hair dyeing systems

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from SCCNFP/0730/03

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:	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

3.3.1.2. Acute dermal toxicity

Taken from SCCNFP/0730/03

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:	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

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Taken from SCCNFP/0730/03

Guideline:	OECD 404 (1981)
Species/strain:	New Zealand albino rabbit
Group size:	2 male, 1 female
Test substance:	MIP 2982, moistened with water
Batch:	028400A8AA
Purity:	87.7%
Dose:	0.5 g
GLP:	In compliance

0.5 g of moistened test substance was applied to 6.25 cm2 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

3.3.2.2. Mucous membrane irritation

Taken from SCCNFP/0730/03

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:	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

3.3.3. Skin sensitisation

Taken from SCCNFP/0730/03

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:	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

3.3.4.	Dermal / percutaneous absorption

Rat and human skin, *in vitro*

Guideline:	OECD 428 (2004)
Tissue:	rat split-thickness skin, 200 µm (2 males, strain: HanBrl: WIST (SPF)) Human split-thickness skin, 200 µm (2 female donors)
Group size:	7 membranes per species
Skin integrity:	permeability coefficient (Kp) of tritium water Kp < 3.5 x 10^{-3} cm/h (rat) Kp < 2.5 x 10^{-3} cm/h (human
Diffusion cell:	automated flow-through cell system, 0.64 cm²
Test substance:	Yellow (MIP 2982)
Batch:	04143CL2 3415121 (radio-labelled, 2109 MBq/mmol, 57 mCi/mmol)
Purity:	91.6% (contains about 8% inorganic salt) > 99% (radio-labelled)
Test item:	Yellow (MIP 2982), as an aqueous solution
Dose:	19 mg/cm²
Dose of test substance:	0.179 mg/cm²
Receptor fluid:	physiological saline, 0.9% NaCl w/v
Solubility receptor fluid:	/
Stability receptor fluid:	/
Method of Analysis:	Liquid Scintillation Counting
GLP:	in compliance
Study date:	February 2005

The ^{14}C-MIP Yellow 2982 [pyridinium-N-methyl-^{14}C] was synthesized (Batch 3415121) to a chemical purity >99% and a specific activity of 2109 MBq/mmol (57 mCi/mmol) or 6251 kBq/mg (169 μCi/mg). Non-radiolabelled Yellow MIP 2982 was from Batch 04143CL2 and had a chemical purity of 91.6% plus about 8% inorganic salts as counter-ions, and <0.1% organic impurities. The dosing solution was prepared as an aqueous dilution of non-labelled Yellow MIP2982 and radiolabelled test item by a procedure that gave [^{14}C]-labelled Yellow (MIP 2982) with a final specific radioactivity of 532 kBq/mg (14.4 μCi/mg). The final dosing solution, adjusted to pH 7.5 with ethanolamine, had a total volume of 1.1 ml and a concentration of 19 mg [^{14}C] Yellow (MIP 2982)/ml.

Full thickness skin was removed from 2 male rats (HanBrl: WIST (SPF)) and stored frozen until prepared for use. Human full thickness skin was obtained post-mortem from the dorsal upper leg of 2 individuals and stored frozen until use. Skin membranes of each species were prepared by removing subcutaneous fat from the full thickness sections and then from the stratum corneal aspect removing the upper 200 μm by dermatome. The membranes were then cut into pieces (ca. 1.8 x 1.8 cm) and mounted in flow-through diffusion cells each consisting of a donor and receptor chamber. The area of skin membrane exposed to the donor chamber was 0.64 cm². From each species 7 membranes in cells were prepared and the cells placed in separate manifolds (one with rat, one with human membranes) and connected to a peristaltic pump. For an equilibration period of 0.5 - 1 hour, saline (0.9% NaCl w/v) was pumped through the receptor chamber at a flow rate of about 3 mL/h.

A target dose level of 0.2 mg/cm² was selected based on the dermal absorption ADME rat study summarized elsewhere in this dossier. A 6 μL aliquot of the dosing solution was applied manually to each skin membrane preparation using a μL-syringe. The amount applied to each cell was shown to be 115 μg/cell or 179 μg/cm² by determination of the radioactivity content of three control doses taken prior to the first, in the middle, and after the last administration for each dose level.

The penetration through the skin membranes was determined over a period of 24 hours under non-occluded conditions. Because of the small surface area of skin available a rinse after 30 minutes was not included in this study. The receptor fluid (physiological saline (0.9% NaCl w/v)) was delivered at a flow rate of about 3 mL/h during the testing period and the perfusate from each cell separately collected at ambient temperature in 1-hour intervals for the 0 to 6 hour period (6 intervals) and in 2-hour intervals for the remaining exposure period (9 intervals).

Twenty-four hours after application the perfusate sampling was terminated and each skin membrane surface rinsed three times with about 0.5 mL ethanol each. All skin membrane rinse fractions were combined according to the individual cells. The skin membranes were removed from the diffusion cell and consecutively stripped until the stratum corneum was removed from the skin membrane, i.e., rat skin membrane 3 to 5 and human skin membrane 7 to 8 tape strips were needed. Up to five consecutive stripping tapes were combined into one specimen and aliquots were measured for radioactivity after solubilisation with tissue solubiliser. The skin membranes remaining after stripping were digested in tissue solubiliser and the radioactivity was determined by LSC. The diffusion cells were then washed with 150 mL ethanol/water (50/50 v/v) and the radioactivity in the cell wash was determined by LSC.

Results
Recovery of radioactivity following application of [^{14}C]- Yellow (MIP 2982) to rat skin membranes:

[% of Dose]									
Dose applied [µg·cm^{-2}]	179								
Cell No.	1	2	3	4	5	6	7	**Mean**	SD
Dislodged Dose									
Membrane Rinse	101.79	103.54	101.29	102.96	101.51	99.75	103.07	**101.99**	1.31
Perfusates									
0-24 h	**0.22**	**0.10**	**0.02**	**0.01**	**0.04**	**0.04**	**0.03**	**0.06**	0.07
Remaining Dose									
Tape Strips I	1.79	3.08	3.27	2.20	1.76	2.58	1.47	**2.31**	0.69
Tape Strips II	-	-	-	-	-	-	-	**-**	-
Subtotal	1.79	3.08	3.27	2.20	1.76	2.58	1.47	**2.31**	0.69
Remaining Skin Membrane	0.41	0.01	0.02	0.04	0.06	0.04	0.22	**0.11**	0.15
Cellwash	0.29	0.14	0.13	0.12	0.07	0.21	0.08	**0.15**	0.08
Recovery	**104.49**	**106.87**	**104.72**	**105.33**	**103.44**	**102.62**	**104.87**	**104.62**	1.36

Recovery of radioactivity following application of [^{14}C]-Yellow (MIP 2982) to human skin membranes:

[% of Dose]									
Dose applied [µg·cm^{-2}]	179								
Cell No.	8	9	10	11	12	13	14	**Mean**	SD
Dislodged Dose									
Membrane Rinse	100.20	97.54	93.14	101.43	113.29	106.50	106.73	**102.69**	6.69
Perfusates									
0-24 h	**< 0.01**	**0.01**	**0.02**	**0.01**	**< 0.01**	**< 0.01**	**0.01**	**0.01**	< 0.01
Remaining Dose									
Tape Strips I	5.94	7.82	9.93	1.43	0.59	0.79	2.42	**4.13**	3.75
Tape Strips II	0.53	0.84	1.20	0.11	0.03	0.12	0.51	**0.48**	0.43
Subtotal	6.47	8.67	11.13	1.54	0.62	0.91	2.93	**4.61**	4.17
Remaining Skin Membrane	0.05	0.13	0.28	0.06	0.03	0.07	0.01	**0.09**	0.09
Cellwash	0.46	0.70	0.86	0.34	0.18	0.19	0.24	**0.42**	0.27
Recovery	**107.20**	**107.03**	**105.43**	**103.38**	**114.13**	**107.68**	**109.92**	**107.82**	3.43

Total absorption and test item recovery is summarized in the next table.

Recovery [% of Dose]		
Skin Membrane:	Rat	Human
Applied Dose [µg/cm2]	179	179
Perfusates	0.066	0.01
Remaining Skin membrane	0.114	0.09
Total Absorbed:	0.18 ± 0.19	0.10 ± 0.09
Skin membrane Rinse	101.99	102.69
Tape Strips	2.31	4.13
Diffusion cell wash	0.15	0.42
Recovery	104.62	107.82

Comparison of the rat and human skin membranes indicates rat skin may be more permeable to Yellow MIP2982 but in both species a 24-hour exposure showed the majority of the test item could be removed by rinsing or was retained in the stratum corneum (tape strips). The apparent 2-fold difference in recovery between rat and human tape strips was considered more attributable to skin rinsing efficiency than to a species difference.

Conclusion

Yellow (MIP 2982), applied as aqueous solution to rat and human skin membranes, penetrated at a low rate. The penetration through rat split-thickness skin membranes was higher than through human split-thickness skin membranes.

Ref.: 1, submission II

Comment
Only 7 chambers were used for each species. Therefore, the amount considered as having penetrated is considered as mean + 2SD. For the rat this is 0.56% (1.0 µg/cm²) of the applied dose and for human skin 0.28% (0.50 µg/cm²) of the applied dose.

Human skin, *in vitro*

Guideline:	OECD 428 (2004)
Tissue:	human dermatomed skin, 400 µm thickness
Group size:	9 membranes from 4 donors (oxidative conditions) 9 membranes from 4 donors (non-oxidative conditions)
Skin integrity:	electrical resistance, > 10 kΩ
Diffusion cell:	glass diffusion cell; 2.54 cm²
Test substance:	Basic Yellow 87
Batch:	04143CL2 3415121 (radio-labelled) (57.0 mCi/mmol)
Purity:	91.6% (UV-Vis spectroscopy) 99% (HPLC)
Test item:	Basic Yellow 87 0.9% (non-oxidative) Basic Yellow 87 1.95% (oxidative)
Dose:	20 mg/cm²
Dose of test substance:	nominal 200 µg/cm² Basic Yellow 87 under oxidative conditions Nominal 200 µg/cm² Basic Yellow 87 under non-oxidative conditions
Receptor fluid:	phosphate buffered saline
Solubility receptor fluid:	40 mg/ml at 20 °C
Stability receptor fluid:	/
Method of Analysis:	Liquid scintillation counting
GLP:	in compliance
Study date:	March –May 2005

Non-radiolabelled test item was from batch 04143CL2 (91.6% pure) and the [^{14}C]-Yellow (MIP2982) from batch 3415121 (radiochemical purity = 99.0%). Human full thickness skin was obtained post-mortem and prepared by dermatome into membranes of average 400 µm (range 200 -500 µm) thickness. Glass diffusion cells were prepared with membranes that were each evaluated for integrity by electrical resistance and those below 10kΩ were excluded from use. Each formulation was tested on 9 membranes receiving 20 mg/cm² of a dosing formulation; after 30 minutes exposure the membranes were each washed and the skin left unoccluded for the remainder of the experimental period. Samples of the phosphate-buffered saline receptor fluid were collected a 9 time points and 24-hours after dose application the exposure was terminated by removing the skin, without rinsing, and then collecting the stratum corneum by tape stripping. The several samples and materials used and collected in the study were evaluated for test item concentrations.

Oxidative conditions

Test Compartment	Amount Recovered ($\mu g_{eq}/cm^2$)												
	Cell 2	Cell 5	Cell 8	Cell 10	Cell 15	Cell 4	Cell 6	Cell 9	Cell 13	Mean	SD	SEM	n
Receptor & Grid	0.071	0.066	0.082	0.068	0.062	0.073	0.072	0.069	0.068	0.070	0.006	0.002	9
Flange	0.002	0.084	0.038	0.112	0.007	0.024	0.048	0.015	0.024	0.039	0.037	0.012	9
Donor Chamber	0.036	0.396	0.141	0.414	0.135	0.197	0.812	0.855	0.304	0.365	0.293	0.098	9
Skin Wash @ 0.5h	171	182	156	162	163	184	169	173	172	170	9.10	3.03	9
Stratum Corneum	0.168	0.417	0.373	0.456	0.221	0.395	0.352	0.323	0.270	0.331	0.095	0.032	9
Remaining Epidermis/Dermis	0.100	0.434	0.463	0.189	0.076	0.391	0.225	0.459	0.387	0.303	0.156	0.052	9
Receptor Fluid	0.012	0.017	0.005	0.002	0.001	0.011	0.011	0.002	0.016	0.009	0.006	0.002	9
Systemically Available*	**0.112**	**0.451**	**0.469**	**0.191**	**0.078**	**0.402**	**0.236**	**0.461**	**0.403**	**0.311**	**0.157**	**0.052**	**9**
TOTAL	171	183	157	163	163	185	170	175	173	171	9.21	3.07	9

Test Compartment	Percent of Dose Recovered (%)												
	Cell 2	Cell 5	Cell 8	Cell 10	Cell 15	Cell 4	Cell 6	Cell 9	Cell 13	Mean	SD	SEM	n
Receptor & Grid	0.042	0.039	0.049	0.040	0.036	0.043	0.043	0.041	0.041	0.042	0.003	0.001	9
Flange	0.001	0.049	0.022	0.066	0.004	0.015	0.028	0.009	0.014	0.023	0.022	0.007	9
Donor Chamber	0.021	0.233	0.083	0.244	0.079	0.117	0.483	0.508	0.180	0.217	0.174	0.058	9
Skin Wash @ 0.5h	101	107	91.8	95.7	96.0	109	100	103	102	101	5.55	1.85	9
Stratum Corneum	0.099	0.246	0.220	0.269	0.130	0.235	0.209	0.192	0.160	0.196	0.056	0.019	9
Remaining Epidermis/Dermis	0.059	0.256	0.273	0.111	0.045	0.232	0.134	0.272	0.230	0.179	0.092	0.031	9
Receptor Fluid	0.007	0.010	0.003	0.001	0.001	0.007	0.007	0.001	0.009	0.005	0.004	0.001	9
Systemically Available*	**0.066**	**0.266**	**0.276**	**0.113**	**0.046**	**0.239**	**0.140**	**0.274**	**0.239**	**0.184**	**0.093**	**0.031**	**9**
TOTAL	101	108	92.4	96.5	96.3	110	101	104	103	101	5.62	1.87	9

* Systemically Available = Sum of Remaining Epidermis and Receptor Fluid data

Non-oxidative conditions

Test Compartment	Amount Recovered ($\mu g_{eq}/cm^2$)												
	Cell 40	Cell 44	Cell 47	Cell 53	Cell 55	Cell 41	Cell 45	Cell 51	Cell 42***	Mean	SD	SEM	n
Receptor & Grid	0.026	0.032	0.023	0.070	0.026	0.064	0.022	0.063	*0.033*	0.041	0.021	0.007	8
Flange	0.050	0.088	0.016	0.103	0.040	0.040	0.096	0.049	*0.133*	0.060	0.031	0.011	8
Donor Chamber	0.279	0.095	0.110	0.577	0.083	0.127	0.056	0.090	*0.358*	0.177	0.175	0.062	8
Skin Wash @ 0.5h	185	189	189	188	189	182	185	199	*187*	188	4.79	1.70	8
Stratum Corneum	1.37	0.451	1.17	0.841	0.380	0.316	0.130	0.085	*0.329*	0.593	0.479	0.169	8
Remaining Epidermis/Dermis	0.497	0.204	0.307	0.656	0.248	0.378	0.176	0.084	*0.413*	0.319	0.186	0.066	8
Receptor Fluid	0.003	0.012	0.019	0.008	0.003	0.014	0.005	0.002	*0.078*	0.008	0.006	0.002	8
Systemically Available*	**0.499**	**0.215**	**0.326**	**0.664**	**0.250**	**0.392**	**0.181**	**0.087**	***0.491***	**0.327**	**0.187**	**0.066**	**8**
TOTAL	188	189	191	191	190	183	186	199	*188*	190	4.62	1.63	8

Test Compartment	Percent of Dose Recovered (%)												
	Cell 40	Cell 44	Cell 47	Cell 53	Cell 55	Cell 41	Cell 45	Cell 51	Cell 42***	Mean	SD	SEM	n
Receptor & Grid	0.014	0.017	0.012	0.037	0.014	0.034	0.012	0.033	*0.018*	0.022	0.011	0.004	8
Flange	0.026	0.046	0.008	0.054	0.021	0.021	0.050	0.026	*0.070*	0.032	0.017	0.006	8
Donor Chamber	0.147	0.050	0.058	0.304	0.044	0.067	0.030	0.048	*0.189*	0.093	0.092	0.033	8
Skin Wash @ 0.5h	97.7	99.4	99.9	99.3	99.6	96.2	97.7	105	*98.5*	99.3	2.53	0.894	8
Stratum Corneum	0.721	0.238	0.616	0.444	0.201	0.167	0.069	0.045	*0.174*	0.312	0.253	0.089	8
Remaining Epidermis/Dermis	0.262	0.107	0.162	0.346	0.131	0.199	0.093	0.044	*0.218*	0.168	0.098	0.035	8
Receptor Fluid	0.001	0.006	0.010	0.004	0.001	0.007	0.002	0.001	*0.041*	0.004	0.003	0.001	8
Systemically Available*	**0.263**	**0.113**	**0.172**	**0.350**	**0.132**	**0.207**	**0.095**	**0.046**	***0.259***	**0.172**	**0.099**	**0.035**	**8**
TOTAL	98.9	99.9	101	100	100	96.7	97.9	105	*99.2*	99.9	2.44	0.862	8

* Systemically Available = Sum of Remaining Epidermis and Receptor Fluid data

*** Cell excluded as profile indicates membrane damage at 0.5h

Values shown in italics are for information purposes only, and are not included in the means

Ref.: 2, submission II

Comment

These were well performed experiments. The amount of Basic Yellow 87 considered as being systemically available is the mean +1SD for each experiment. Therefore, under oxidative conditions, 0.47 μg/cm² (0.28%) of Basic Yellow 87 was absorbed from a formulation containing 0.975% of it; under non-oxidative conditions, 0.51 μg/cm² (0.27%) of Basic

Yellow 87 was absorbed from a formulation containing 0.9% of it. Since a maximum concentration of 1% was applied for the latter value was corrected accordingly (non-oxidative absorption 0.57 µg/cm^2) Within these absorbed doses of Basic Yellow 87 0.315 µg/cm^2 (oxidative) and 0.38 µg/cm^2 (non-oxidative conditions) of the relevant cation are absorbed. These values may be used in calculating the MOS.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral toxicity

Taken from SCCNFP/0730/03

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:	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; behavioural 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

3.3.5.2. Repeated Dose (14 days) dermal

Taken from SCCNFP/0730/03

Guideline:	OECD 402 (1987)
Species/strain:	Himalayan spotted guinea pigs
Group size:	4 males, 4 females
Test substance:	MIP 2982
Batch:	CGF-FO16737/0016
Purity:	>92%
Dose levels:	0.1 ml/7 cm², 4 concentrations 0.5, 1, 3 and 5%

GLP: In compliance

Two applications sites of 7 cm² 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

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

Taken from SCCNFP/0730/03

Guideline:	OECD 408 (1981)
Species/strain:	Wistar rat SPF-bred
Group size:	10 males + 10 females
Test substance:	MIP 2982 in feed
Batch:	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 biochemistry 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. This corresponds to a does of 6.76 mg/kg bw/day of the cation of Basic Yellow 87, which represents the relevant toxic principle.

Ref.: 5

3.3.5.3. Chronic (> 12 months) toxicity

No data submitted

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial Reverse Mutation Test, re-evaluated

Guideline:	OECD 471	
Species/strain:	*S. typhimurium*, TA98, TA100, TA1535, TA1537, *E. coli* WP2 *uvrA*	
Replicates:	Triplicate plates, 2 independent experiments	
Test substance:	MIP Yellow 2982	
Solvent:	water	
Batch:	028400A8AA	
Purity:	87.7%	
Concentrations:	*experiment 1*:	33.3, 100, 333, 1000, 2000 and 3330 µg/plate without S9-mix 33.3, 100, 333, 1000, 3330 and 5000 µg/plate with rat S9-mix 33.3, 100, 333, 1000, 3330 and 5000 µg/plate with hamster S9-mix
	experiment 2:	33.3, 100, 333, 1000, 2000 and 3330 µg/plate without S9-mix 33.3, 100, 333, 1000, 2000, 3330 and 5000 µg/plate with rat S9-mix 33.3, 100, 333, 1000, 2000, 3330 and 5000 µg/plate with hamster S9-mix
Treatment:	pre-incubation method with 20 minutes (without S9-mix or with rat S9-mix) or 30 minutes (hamster S9-mix) pre-incubation and 52 ± 4 h incubation	
GLP:	In compliance	
Study period:	23 October 2001 – 17 December 2001	

MIP Yellow 2982 has been investigated for its ability to induce gene mutation in *S. typhimurium* and *E. coli* using the preincubation method both with and without S9-mix. Liver S9 fraction from Aroclor 1254-induced rats or from uninduced Golden Syrian hamsters was used as exogenous metabolic activation system. Test concentrations were based on the results of a dose rangefinding assay with strains TA100 and WP2*uvr*A. Toxicity was evaluated for 10 concentrations up to the prescribed maximum concentration of 5000 µg/plate on the basis of a reduction in the number of revertant colonies and/or clearing of the bacterial background lawn. Negative and positive controls were in accordance with the OECD guidelines.

Results
In the dose range finding assay, inhibition of growth, as evidenced by a decrease in revertant frequency or thinning of the background lawn was observed in TA100 at doses ≥ 667 µg/plate in the presence of S9-mix and at doses ≥ 100 µg/plate in the absence of S9-mix and in WP2*uvr*A at doses ≥ 1000 µg/plate in the presence of S9-mix and at doses ≥ 667 µg/plate in the absence of S9-mix
In experiment 1, in the absence of activation and in the presence of rat S9-mix, no dose related and biologically relevant increase in revertant numbers was observed, in any tester strains used. In the presence of hamster liver S9-mix: an increase in revertant numbers

was observed for both a frameshift (TA1537) and a base-pair substitution strain (*E. coli*) at 1000 µg/plate. In TA1537 the number of revertants at 1000 µg/plate was 49 ± 5 and 33 ± 7; in *E. coli*, which is outside the historical negative control values for both tester strains. Although, the increases are not concentration related it should be noted that at higher concentrations cytotoxicity was observed that could have prevented the expression of increased mutant frequencies. For the other strains, no statistically or biologically relevant increase of mutant frequencies has been observed as compared to the controls. Positive controls showed the expected response.
In a confirmatory test (experiment 2) an additional concentration of 2000 µg/plate, which was between the concentration with a slight positive effect (1000 µg/plate) and the next concentration (3330 µg/plate), which was toxic. The slightly positive effect with hamster liver S9-mix could not be confirmed, and it is therefore concluded that MIP Yellow 2982 did not induce gene mutations in bacteria.

Conclusions
Based on the reversion rate, and under the conditions of the 2 experiments performed in the presence of "normal" and "reductive" S9-mix, it is concluded that the test agent MIP Yellow 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 V79 cell line (mutation at the *hprt* locus)	
Replicates:	2 independent tests	
Test substance:	MIP 2982	
Batch:	CGF-F016737/0013	
Purity:	88.6% (HPLC)	
Vehicle:	culture medium	
Concentrations:	experiment I:	3, 10, 30, and 100 µg/ml without S9-mix
		3, 30, 100, and 300 µg/ml with S9-mix
	experiment II:	3, 30, 100 and 200 µg/ml without S9-mix
		30, 50, 100, 300, 450 and 600 µg/ml with S9-mix
Treatment	4 h both without and with S9-mix; expression period 72 h and a selection period of 7 days.	
GLP:	In compliance	
Study period:	7 July 1997 – 10 September 1997	

MIP 2982 has been investigated for gene mutation at the *hprt* locus in V79 Chinese hamster cells in the presence or absence of an activation system. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. Test concentrations were based on the results of a XTT assay. In this pre-test on cytotoxicity 8 concentrations ranging from 3 up to 1000 µg/ml were used.
In the main test, cells were treated for 4 h followed by an expression period of 72 h to fix the DNA damage into a stable *hprt* mutation. Toxicity was measured as cloning efficiency of the treated cultures relative to the cloning efficiency of the solvent control cultures. Negative and positive controls were in accordance with the OECD guideline

Results
In experiment I, the mutant frequencies of all concentrations tested both in the absence or presence of activation 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. In addition, no dose-response trend was noted.

In experiment II, no statistically or biologically significant increase in mutant frequency was observed over the concurrent solvent controls for any concentration tested both in the absence or presence of activation.

Conclusion
Under the experimental conditions used, MIP 2982 was considered not mutagenic in this *hprt* gene mutation assay in V79 cells.

Ref.: 15

In vitro Mammalian Cell Gene Mutation Test

Guideline:	OECD 476 (1998)	
Species/strain:	mouse lymphoma L5178Y cell line	
Replicates:	duplicate cultures, 2 independent experiments	
Test substance:	Yellow MIP 2982	
Batch:	04143CL2	
Purity:	91.6%	
Vehicle:	deionised water	
Concentrations:	experiment I:	118.8, 237.5, 475.0, 712.5, 950.0 µg/mL without S9-mix 59.4, 118.8, 237.5, 356.3, 475.0, 712.5 µg/mL with S9-mix
	experiment II:	200, 300, 400, 500, 600 µg/mL without S9-mix: 30, 45, 60, 90, 120 µg/mL with S9-mix:
Treatment:	4 h treatment with and without S9-mix (rat and hamster in experiment I; hamster in experiment II), expression period 72 h and a selection period of 10-15 days.	
Positive control:	methyl methane sulfonate without metabolic activation cyclophosphamide with metabolic activation (rat liver) N-nitrosodimethylamine with metabolic activation (hamster liver)	
GLP:	in compliance	
Study date:	17 August 2004– 25 October 2004	

Yellow MIP 29 was investigated for the induction of gene mutations in the mouse lymphoma assay. Two main experiments were performed with and without metabolic activation.
Liver S9 fraction from phenobarbital/β-naphthoflavone-induced rats or from uninduced Golden Syrian hamsters was used as exogenous metabolic activation system. The concentrations used in the main experiments were based on toxicity in a preliminary test and in the first main test. No 24 h treatment was used in the second experiment because a mutagenic effect was observed in the first experiment with 4 hours treatment. Relevant positive controls were included according to guidelines.

Results
In both experiments in the absence and presence of S9-mix the appropriate level of toxicity (10-20% survival after the highest concentration) was reached. In the first experiment relevant toxicity (relative cloning efficiency I or relative total growth below 50%) was detected at 475.0 µg/mL and above without metabolic activation and at 118.8 µg/mL and above with metabolic activation (rat S9). Using hamster S9 toxic effects occurred already at concentrations as low as 59.4 µg/mL.
There was a clear concentration dependent increase in mutant frequency both without and with S9-mix. The genotoxic potency was highest with metabolic activation and similar for rat and hamster S9-mix. The ratio of small versus large colonies was shifted towards small colonies, indicating a clastogenic effect.

Conclusion

Under the experimental conditions used, Yellow MIP 29was mutagenic and/or clastogenic in mouse lymphoma cells both with and without metabolic activation. The shift of the ratio of small versus large colonies towards small colonies indicates to a clastogenic effect.

Ref.: 3, submission II

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	
Vehicle:	cell culture grade water	
Batch:	028400A8AA	
Purity:	90.5% (HPLC)	
Concentrations:	initial experiment:	33.9, 48.4, 69.1, 98.7 µg/ml without S9-mix 69.1, 98.7, 202.0, 288.0 µg/ml with S9-mix
	confirmation experiment:	3.55, 7.10, 14.2 µg/ml without S9-mix 57.8, 92.8, 155.0, 206.0 µg/ml with S9-mix
Treatment:	initial experiment:	3 h without and with S9-mix; harvest time 22 h after start of treatment.
	confirmation experiment:	22 without S9-mix; harvest time 22 h after start of treatment. 3 h with S9-mix; harvest time 22 h after start of treatment.
GLP:	In compliance	
Study period:	4 October 2001 – 7 December 2001	

MIP 2982 in water has been investigated for induction of chromosomal aberrations in human lymphocytes withdrawn from healthy non-smoking donors. 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. Cells were treated for 3 h and harvested 22 h after the start of treatment or for 22 h (confirmatory experiment without S9-mix only) and harvested immediately after the end of treatment. Approximately 2 h before harvest, each culture was treated with Colcemid® (final concentration 0.1 µg/ml) to block cells at metaphase of mitosis. Chromosome (metaphase) preparations were examined microscopically for chromosomal aberrations and the mitotic index. Negative and positive controls were in accordance with the OECD guideline

Results

Both in the initial and confirmatory experiment, a biologically relevant increase in the number of cells with chromosomal aberrations, polyploidy or endoreduplication was not found at any concentration tested neither without nor with S9-mix.

Since an excessive cytotoxicity was observed in one of the cultures treated with 98.7 µg/ml (MI = 0.1 %),all the required 200 cells were scored from the duplicate culture. This deviation does not alter the validity of the test nor the results.

Conclusion

Under the experimental conditions used, MIP 2982 was not genotoxic (clastogenic) in this chromosomal aberration test with human lymphocytes either in the absence or in the presence of S9-mix.

Ref.: 14

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

Taken from SCCNFP/0730/03, re-evaluated

Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474 (1983)
Species:	NMRI mice
Group sizes:	6 male and 6 female mice
Material:	MIP 2982
Batch:	CGF-F016737/0013
Purity:	88.6% (HPLC)
Vehicle:	deionized water
Dose levels:	0, 12.5, 40.0 and 125 mg/kg bw
Route:	oral gavage
Sacrifice times:	24 h and 48 h (top dose only) after treatment.
GLP:	In compliance
Study period:	18 August 1998 – 11 February 1999

MIP 2982 has been investigated for induction of micronuclei in the bone marrow cells of male and female mice. Dose levels were based on the results of a preliminary toxicity test in male and female mice on toxic signs and mortality recorded over a period of 48 h. In the main experiment mice were exposed orally to 0, 12.5, 40.0 and 125 mg/kg bw/day. The substance was administered by a single intragastric gavage and the animals were sacrificed 24 and 48 hours (top dose only) after administration. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatic erythrocytes (PCE/NCE). Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-experiment for toxicity all mice died after 1000 mg/kg bw, 2 males and 1 female mouse after 500 mg/kg bw and 2 male mice after 250 mg/kg bw. According to clinical observations and toxic reactions of the mice, the top dose has been chosen to be 125 mg/kg bw. The clinical observations included reduction of spontaneous activity, eyelid closure and apathy. All treated mice had yellow coloured urine.
A decrease in the PCE/NCE ratio was not observed at both sampling times; this reflects the lack of cytotoxicity of MIP 2982. However, the clinical signs reported and the yellow coloured urine indicate systemic distribution and thus bioavailability of MIP 2982.
Biologically relevant increases in the number of micronucleated polychromatic erythrocytes over the concurrent vehicle control values were not observed at both sampling times nor for any dose level tested.

Conclusions
Under the experimental conditions used MIP 2982 did not induce an increase in the number of bone marrow cells with micronuclei and, consequently, MIP 2982 is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 17
Ref.: 4, submission II

Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells

Guideline:	OECD 486
Species/strain:	Wistar rat, HanIbm: WIST (SPF) strain
Group size:	4 male rats
Test substance:	MIP Yellow 29
Batch:	CGF-F016737/0013
Purity:	88.6% (HPLC)
Vehicle:	deionised water
Dose levels:	0, 250 and 500 mg/kg bw.
Route:	orally
Exposure time:	2 h and 16 hours post-treatment
GLP:	In compliance
Study period:	13 October 1998 – 18 February 1999

MIP Yellow 29 was investigated for the induction of unscheduled DNA synthesis (UDS) in hepatocytes of rats. Test concentrations were based on a pre-experiment for toxicity measuring acute toxic symptoms at intervals of 1 h and 24 h after oral administration of 1000, 750 and 500 mg/kg bw. In the main experiment the highest dose was 500 mg/kg bw. The animals were starved over night before treatment.
Hepatocytes for UDS analysis were collected by perfusion with 0.05% w/v collagenase approximately 2 h and 16 h after administration of MIP Yellow 29. The quality of the actual performed perfusion was determined by the trypan blue dye exclusion method. Three cultures were established for each animal. At least 90 minutes after plating the cells were incubated for 4 h with 5 µCi/ml ^{3}H-thymidine (specific activity 20 Ci/mmol) followed by overnight incubation with unlabelled thymidine. Evaluation of autoradiography was done after 18 days.
UDS was reported as nuclear and cytoplasmic grain counts as well as the net grain counts (nuclear minus cytoplasmic grains). Unscheduled synthesis was determined in 50 randomly selected hepatocytes on 2 replicate slides per rat. Negative and positive controls were in accordance with the OECD guideline.

Results
In the pre-experiment for toxicity at 1000 mg/kg bw one rat died within 1 h after treatment. Clinical signs at 750 mg/kg bw (reduction of spontaneous activity, ruffled fur and apathy) were estimated too close to the maximum tolerated dose. Therefore 500 mg/kg bw was selected as the highest dose. At this dose the animals still showed abdominal position, apathy, tremor and dyspnoea. The mice recovered however, within 1 day. The urine of all treated mice coloured orange, indicating to systemic availability of MIP Yellow 29.
The viability of the hepatocytes was not substantially affected by the treatments. Treatment with MIP Yellow 29 at doses of 250 and 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.

Conclusion
Under the experimental conditions used, MIP Yellow 29 did not induce unscheduled DNA synthesis and, consequently, is not genotoxic in rats in the *in vivo* UDS test.

Ref.: 16

3.3.7. Carcinogenicity

No data submitted

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted

3.3.8.2. Teratogenicity

Taken from SCCNFP/0730/03

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:	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

3.3.9. Toxicokinetics

Bioavailability after oral administration, mice

Guideline:	OECD 417 (1984)
Species/strain:	Hybrid mice, NMRI, SPF-quality
Group Size:	15 females
Test material:	Yellow (MIP 2982)
Batch:	04143CL2 (non radiolabelled) 3415121 (radio-labelled, 2109 MBq/mmol; 57 mCi/mmol)
Purity:	91.6% (non radiolabelled, containing about 8% inorganic salts, organic impurities < 0.1%) > 99% (radio-labelled)
Vehicle:	water (MilliQ)
Dose:	40 mg/kg bw
Administration:	oral, single administration by gastric intubation
GLP:	in compliance
Study period:	August 2004 –April 2005

This study was designed in order to investigate the bioavailability of B117 after oral exposure in order to support the results of the micronucleus tests.

B117 was orally administered to 15 female mice at the dose level of 40 mg/kg bw. 0.5, 1, 2, 4 and 24 hours after administration, 3 mice were sacrificed after anaesthesia with carbon dioxide and the concentration of the tested compound was determined in plasma and femur. No other tissues or endpoints were examined from these animals.

B117 was rapidly absorbed from the gastro intestinal tract and the maximum concentration in plasma was observed 0.5 hours after exposure and corresponded to 4.823 ppm (µg B117 equivalents/g). Then a two phase decrease of the plasmatic concentration was observed with an initial half life of 1.2 hours and a second half life of 6 hours. Within 24 hours after exposure, almost all B117 was removed from plasma and the concentration in plasma decreased to 0.071 ppm. The AUC_{0-24h} for plasma was 14.25 µg.h/g +/- 3.48.

The maximum concentration in femur was observed at 0.5h accounting for 1.273 ppm equivalents/g. The depletion kinetics in femur was similar that observed in plasma, but a little bit slower with a initial half life of 3.3 hours and a terminal half life of 13 hours. Within 24 hours after administration, the concentration in femur decreased to 0.203 ppm. It is assumed by the authors that the radioactivity determined in femur is predominately located in bone marrow and that it is correlated to the unchanged test item or its metabolites.

The authors concluded that B117 orally administered to mice results in a rapid and direct exposure of the bone marrow and the associated cellular components to the tested compound.

Ref.: 5, submission II

Comments
This study gives evidence that B117 orally administered at the dose of 40 mg/kg bw reached the bone marrow and the target cells investigated in the micronucleus test.

ADME after oral and dermal administration, rat

Guideline:	OECD 417 (1984)
Species/strain:	rat, HanBrl:WIST (SPF): Wistar rats, outbred, SPF-quality
Group Size:	17 females (oral: 9; dermal: 8)
Test material:	Yellow (MIP 2982)
Batch:	04143CL2 (non radio-labelled) 3415121 (radio-labelled, 2109 MBq/mmol; 57 mCi/mmol)
Purity:	91.6% (non radio-labelled containing about 8% inorganic salts, organic impurities < 0.1%) > 99% (radio-labelled)
Vehicle:	water
Dose:	10 mg/kg bw (oral administration by gastric intubation) 0.2 mg/cm² (dermal administration on the dorsal area 10 cm^2)
Exposure period:	30 minutes (dermal administration)
GLP:	in compliance
Study period:	June 2004 – April 2005

This study was designed in order to investigate the absorption, distribution, metabolism and excretion of B117 after oral and dermal exposure in female rats.

B117 was orally and dermally administered to two groups of female rats (9 for oral exposure and 8 for dermal exposure) at the dose level of 10 mg/kg bw or 0.2 mg/cm² respectively. The concentration of radioactivity was determined in urine, faeces, blood, plasma and organs/tissues at different time points after administration.

After 30 minutes dermal application, a skin wash and a skin stripping were performed to remove the remaining test item and stratum corneum from the application site. Skin wash and skin strips were sampled for determining the amount of test item remaining.

3 female rats were sacrificed at 24, 48 and 96 hours after oral administration and 4 female rats at 24 and 96 hours after dermal administration.

The results show that after oral administration at the dose of 10 mg/kg bw, 6% of the administered test item was absorbed from the gastrointestinal tract into the systemic circulation. The oral absorption was fast with a maximum concentration in blood and plasma reached 1 hour after administration and accounting for 0.143 ppm and 0.283 ppm. Then a two phase decrease of the concentration was observed with an initial half life of 7.5 and 5.6 hours in blood and plasma respectively and a second half life of 48 hours (blood) and 45 hours (plasma). Within 96 hours after exposure, almost all B117 was removed from blood and plasma and the concentration decreased to values below or very closed to LOQ, i.e. 0.004 ppm (B117 equivalent). The $AUC_{0\text{-}24h}$ for blood was 1.72 µg.h/g +/- 0.13 and 2.10 +/- 0.24 for plasma.

The excretion data show that the orally administered B117 was rapidly excreted predominately with faeces (89% of the dose was excreted in faeces after 96 hours versus 5.3% in urine). Only 0.1% of the dose was still remaining in tissue and carcass after 96 hours. The highest residue levels were found at 24 hours after oral administration in liver and kidney but due to the very low absorption were very low.

Metabolites were examined by HPLC analysis of urine and faeces extracts and revealed a simple metabolite pattern. The metabolite pattern in urine revealed 1 major and 10 minor metabolites fractions. The major fraction represented more than 50% of the radioactivity in urine or 2.5% of the dose and was shown to contain a glucuronic acid conjugate of B 117 formed after hydroxylation of the phenyl moiety and its structural isomer.

The results show that after dermal administration at the dose of 0.2 mg/cm² on the dorsal area of female rats (10 cm^2) during 30 min followed by a skin washing, a very low fraction of the applied dose was absorbed from the skin into the systemic circulation, around 0.3 % after 30 min of exposure and remained constant within 96 hours. The concentrations of radioactivity for all blood sampling time point were below the limit of quantification. The amount of radioactivity determined in the stratum corneum was almost constant during the experimental period of 5 days, accounting for 2.56 and 2.79% of the dose.

Conclusion
The high extent of faecal excretion, mainly as unchanged B117 confirms the low extent of absorption after oral exposure. After dermal exposure, B117 was also poorly absorbed.
Ref.: 6, submission II

Comments
Due to the low absorption of B117 after oral exposure, the NOAEL of 10 mg/kg bw/d derived from the 13 week oral study in rats should be adjusted by a factor of 10 to be used in the MOS calculation.
Species difference between mouse and rats was noted. Consequence to be further elaborated.

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

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:	CGF-FO16737/0016
Dose levels:	0.025 ml/cm² 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:	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

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Basic Yellow 87
(non-oxidative conditions)

Absorption through the skin	**A (mean + 1SD)**	**=**	**0.38 (cation) µg/cm²**
Skin Area surface	**SAS (cm^2)**	**=**	**580 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.22 mg**
Typical body weight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.0037 mg/kg**
No Observed Adverse Effect Level bw (sub-chronic toxicity, oral, rats)	**NOAEL**	**=**	**6.76 (cation) mg/kg**
Adjustment for 10% bioavailability	**NOAEL adjusted**	**=**	**0.676 mg/kg bw**
MOS		**=**	**184**

The MoS under oxidative conditions is very similar.

3.3.14. Discussion

Physico-chemical specification
Basic Yellow 87 is used in oxidative hair dyeing systems and in non-oxidative hair dyeing systems.
The identity, purity and the impurities of those batches and formulations of Basic Yellow 87 which have been used for toxicological testing have sufficiently been documented.
The same is true for the stability and homogeneity of most of the test formulations which are relevant for the opinion.
There are no data about the stability of Basic Yellow 87 in hair dye formulations.
All batches analysed contain (mono)methyl sulphate (7.4 to 35.7%), which is used as an anion to the dye. No specific toxicity data for monomethyl sulphate were provided. However, the toxicity testing was performed in the presence of this anion.

General toxicity
In an acute oral toxicity study in rats, the observed median lethal dose was between 500 and 1000 mg/kg bw in female and > 1500 mg/kg bw in males. In an acute dermal toxicity study in rats, no signs of toxicity were observed at the dose tested of 2000 mg/kg applied for 24 hours to an area of 10% of the total surface area.

In an oral 28-day study in rats, the No Observed Adverse Effect Level (NOAEL) was 174 mg/kg bw/day, the highest dose tested and the No Observed Effect Level (NOEL) was 39 mg/kg bw/day based on reduced body weight and food intake and reduced total protein and globulin level.

In a dermal 14-day study in rats, no effects were reported.

In an oral 90-day study in rats, the NOAEL was 10 mg/kg bw/day based on some biochemical modifications. Due to the low oral bioavailability of B117 as shown in an ADME study in rats after oral and dermal administration, the NOAEL of 10 mg/kg bw/day was adjusted by a factor of 10 to be used in the MOS calculation and corrected to the cation content.

No two generation reproduction toxicity study was submitted.
In a teratogenicity study, the NOAEL of maternal and foetal toxicity was 60 mg/kg bw/day based on reduced food intake and body weight in the dams.

Irritation, sensitisation
No findings of erythema or oedema were noted in any of the animals. The test article was non-irritating to rabbit skin. It was moderately irritating to rabbit eyes under conditions of this study.
None of the animals showed any reaction. Under the test conditions, Basic Yellow 87 was not a sensitiser.

Dermal absorption
Rat and human skin *in vitro*: only 7 chambers were used for each species. Therefore, the amount considered as having penetrated is considered as mean + 2SD. For the rat this is 0.56% (1.0 µg/cm²) of the applied dose and for human skin 0.28% (0.50 µg/cm²) of the applied dose.

Human skin *in vitro*: the amount of Basic Yellow 87 considered as being systemically available is the mean +1SD for each experiment. Therefore, under oxidative conditions, 0.47 µg/cm² (0.28%) of Basic Yellow 87 was absorbed from a formulation containing 0.975% of it; under non-oxidative conditions, 0.51 µg/cm² (0.27%) of Basic Yellow 87 was absorbed from a formulation containing 0.9% of it. Since a maximum concentration of 1% was applied, the latter value was corrected accordingly (non-oxidative absorption 0.57 µg/cm^2) Within these absorbed doses of Basic Yellow 87 0.315 µg/cm^2 (oxidative) and 0.38 µg/cm^2 (non-oxidative conditions) of the relevant cation are absorbed. These values (0.315 µg/cm² (oxidative) and 0.38 µg/cm² (non-oxidative), cation content, corrected for anion content) may be used in calculating the MOS.

MOS calculation
In the studies used for the MOS calculation different batches with highly varying methylsulphate contents were used (35.7% dermal absorption study; 7.5% 90-day study). The results were therefore adjusted to the cation content.

Mutagenicity

Basic Yellow 87 has been tested for the 3 genetic endpoints (gene mutations, structural and numerical chromosomal aberrations). The substance did not induce gene mutations in bacteria either without metabolic activation system or with standard or reductive metabolic activation system. The test substance did not induce gene mutations in mammalian cells at the *hprt* locus either in the absence or in the presence of standard metabolic activation system. Basic Yellow 87 induced gene mutations and/or chromosomal aberrations in the mouse lymphoma assay without metabolic activation and both with standard and reductive metabolic activation systems, with a similar potency for the two activation systems. No clastogenic effect was observed in human lymphocytes either in the absence or in the presence of standard metabolic activation.
The genotoxic activity observed *in vitro* was not confirmed in two *in vivo* tests. Negative results were obtained in the mouse micronucleus assay. The systemic availability of Basic Yellow 87 in the study observed by clinical signs and coloured urine, was confirmed in an oral toxicokinetic study giving evidence that Basic Yellow 87 administered orally at a dose of 40 mg/kg bw reached the bone marrow and thus the target cells of the micronucleus test. Also an *in vivo/in vitro* UDS assay on rat hepatocytes is negative.
As the positive *in vitro* results were not confirmed in *in vivo* tests, Basic Yellow 87 can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No data submitted

4. CONCLUSION

Based on the data provided, the SCCS is of the opinion that Basic Yellow 87 does not pose a risk to the health of the consumer when used in non-oxidative and oxidative hair dye formulations up to a concentration of 1.0% on-head.

5. MINORITY OPINION

Not applicable

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