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

CONCERNING

HC YELLOW NO. 7

COLIPA n° B80

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

1. Terms of Reference

1.1. Context of the question

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

1.2. Request to the SCCNFP

The SCCNFP is requested to answer the following questions:

* Is HC Yellow No. 7 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

HC Yellow No. 7 (INCI name)

2.1.2. Chemical names

Chemical name	:	1-(4'-Aminophenylazo)-2-methyl-4-(bis-2-hydroxyethyl)aminobenzene
CAS name	:	Ethanol, 2',2'-[[4-[(4-aminophenyl)azo]-3-methylphenyl]imino]bis-
Synonyms	:	4'-Aminophenyl-6-methyl-4-N,N-(di-β-hydroxyethylamino)azobenzene
	:	2-[[4-(4-Amino-phenylazo)-3-methyl-phenyl]-(2-hydroxy-ethyl)-amino]-ethanol

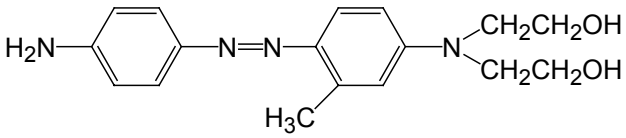
2.1.3. Trade names and abbreviations

Trade name	:	IMEXINE® AA (Chimex)
COLIPA No.	:	B-80

2.1.4. CAS No. / EINECS No.

CAS No.	:	104226-21-3
EINECS No.	:	/

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. formula	:	$C_{17}H_{22}N_4O_2$
Mol. weight	:	314.4

2.1.7. Purity, composition, and substance codes

All analytical data relate to Batch Op. 56

Purity

titre as determined by potentiometry	:	98-100%
water content	:	2%
ash content	:	1.8%

Potential impurities and reaction intermediates

N-(4-amino-phenyl)-acetamide (Impurity A)	:	< 50 ppm
2-[(2-hydroxy-ethyl)-m-tolyl-amino]-ethanol (Impurity B)	:	< 500 ppm
N-(4-{4-[bis-(2-hydroxy-ethyl)-amino]-2-methyl-phenylazo}-phenyl)-acetamide (Impurity C)	:	< 500 ppm

Three other impurities were detected by HPLC and their identity investigated by combined HPLC-MS: however, these compounds remain unidentified.

Solvent residues

ethanol	:	< 25 ppm
iso-propanol	:	< 25 ppm
n-propanol	:	< 50 ppm

Other

potassium ions	:	0.8%
acetic acid	:	1.6%
heavy metals	:	< 10 ppm

2.1.8. Physical properties

Appearance	:	Orange crystalline powder; odourless
Melting point	:	148-152 °C; 149 °C
Boiling point	:	/
Density	:	0.18 g/cm^3
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{OW}	:	2.3 (calculated); 2.6 (at 24 °C; Method A8, Directive 84/449/EEC)
Storage	:	Protect sealed from light and moisture

HPLC and HPTLC procedures and features provided. IR, UV-Vis, MS, and NMR spectral characteristics also available for identification purposes.

2.1.9. Solubility

Water	:	0.035 g/l at 20 °C (method A6, Directive 84/449/EEC)
Ethanol 95%	:	soluble at 1 %.
Receptor fluid*	:	≥ 15.9 µg/ml at 32 °C

* receptor fluid used in percutaneous absorption study : Instamed® PBS buffer w/o Ca^{2+}, Mg^{2+} 9.55g/l containing 0.25% of Tween 80

General comments on analytical and physico-chemical characterisation

* Chromatographic purity of the compound has not been reported.

* Purity of the chemical reported for one batch only (Batch Op. 56). Data on impurities and their range of concentration (in more than one batch) are needed.

* Potential impurities, deriving from reagents and/or intermediates, were not quantitated due to the rather high limits of detection. Three trace unknowns were detected in the only one batch reported. Related health hazards unknown or not addressed.

* Chemical purity not stated in many toxicity study reports.

* No experimental data on stability provided. However, chemical sensitivity to light and moisture was implied.

2.2. Function and uses

HC Yellow No. 7 will be incorporated into semi-permanent and temporary hair dyeing lotions at a maximum concentration of 0.25%. It is common practice for 35 ml of undiluted formulation to be applied for a period of 30 minutes before washing.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Guideline	:	OECD 401 (1981)
Species/strain	:	Sprague Dawley rats, HC/CFY strain (remote Sprague Dawley)
Group size	:	5 males + 5 females
Test substance	:	HC Yellow No. 7 suspended in 1.0% aqueous methylcellulose
Batch No.	:	DG 3
Purity	:	/
Dose	:	640, 800, 1000, and 1600 mg/kg bw
Observ. period	:	14 days
GLP	:	In compliance

Groups of 5 male and 5 female rats received a single dose of test substance at 640, 800, 1000, and 1600 mg/kg bw by gastric gavage. The animals were observed daily for 14 days and body weights were recorded on days 1, 8, and 15 of the study. Macroscopic examination of main organs was performed after autopsy. No histological examinations were performed.

Results

Mortalities occurred within 3 days of dosing at 800 mg/kg bw and above (1, 2, and 3 females, and 1, 3, and 3 males at 800, 1000, and 1600 mg/kg bw, respectively). Clinical signs included piloerection, diuresis, abnormal body posture and gait at all dose levels. With increasing dose, increased salivation and diarrhoea, lethargy, and decreased respiratory rate were reported. At autopsy congestion of the lungs was reported in rats treated with doses of 800 mg/kg bw and above. The LD_{50} was reported to be 1306 mg/kg bw for males and 1203 mg/kg bw for females.

Ref. : 1

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

Guideline	:	OECD 407 (1981)
Species/strain	:	Sprague Dawley rats. Crl : CD(SD) BR
Group size	:	10 males + 10 females
Test substance	:	IMEXINE AA suspended in 0.5% aqueous carboxymethylcellullose
Batch No.	:	Op. 26
Purity	:	99.9 %
Dose	:	0, 25, 80, and 250 mg/kg bw/day
Exposure period	:	29/30 days (7 days per week)
GLP	:	In compliance

Groups of 10 male and 10 female rats were dosed with the test substance by gavage at 0, 25, 80, and 250 mg/kg bw/day, 7 days a week for 29/30 days. The dosing solutions were analysed at the beginning and end of the study for stability and verification of homogeneity and concentration. During the study, the animals were observed daily for clinical signs and mortality, and weekly for body weight and food consumption. During week 4, urine was collected overnight for urinalysis and blood was sampled from the orbital sinus for haematology and blood biochemistry. At the end of the treatment period, a full autopsy was conducted with recording of weights and macroscopic and microscopic examination of major organs. Ophthalmoscopy was conducted on control and high dose animals before the start of the study and at the end of the treatment period on all surviving animals.

Results

Two premature deaths were reported in the high dose male group, on days 9 and 27. Clinical signs preceding death included yellow discoloration of the extremities, hair and urine. Microscopically, kidney changes were noted but the cause of death was not established.

Yellow to orange staining of the urine was seen in all animals at 80 and 250 mg/kg bw/day, with staining of the fur and extremities in all males and in females dosed at 250 mg/kg bw/day. Hypersalivation, swollen head and/or piloerection were observed in a small number of animals at 250 mg/kg bw/day.

The mean body weight gain was not affected by the compound at 25 and 80 mg/kg bw/day. There was a slight decrease in the mean body weight gain in males at 250 mg/kg bw/day (-10% in week 2 and -5% in week 3). Food consumption for all treated groups was comparable to controls. All animals dosed at 250 mg/kg bw/day, and all males and 7/10 females at 80 mg/kg bw/day showed a bilateral yellow coloration of the fundus of the eye. No changes were observed at 25 mg/kg bw/day.

Slight changes in haematological parameters were within the range of historical controls and not dose-related. Increases in aspartate aminotransferase, alanine aminotransferase, total bilirubin, and triglycerides were reported in animals treated with 250 mg/kg bw/day. Other minor changes in biochemical parameters were within the normal range and not considered to be of

toxicological importance. Orange or dark orange coloration of the urine was reported in the males at 25 mg/kg bw/day and in all animals at 80 and 250 mg/kg bw/day. No other changes were reported for the urinalysis.
Macroscopic findings were staining of the skin and tail, observed in animals dosed with 80 and 250 mg/kg bw/day. Liver enlargement was seen in one male at 250 mg/kg bw/day. A slight to moderate increase in the absolute and relative liver weight (15% in males, 30-35% in females) and relative kidney weight (14% in males, 11% in females) was noted at the highest dose in both sexes. There was also an increase in absolute (25%) and relative (37%) weight of adrenals in high dose males. There were no significant changes in organ weights at lower doses. Tubular necrosis with a yellowish pigment accumulation and a slight to marked tubular basophilia was reported in 2/10 males at 250 mg/kg bw/day. Cell hypertrophy (diffuse and/or centrilobular) was reported in some animals of both sex at the highest dose, in line with the increase in the liver weights and enlargement. The authors suggested that the hepatic hypertrophy was in response to an increased functional demand. Multifocal myopathy was seen in the skeletal muscle in some animals of both sexes at 250 mg/kg bw/day.
Administration of HC Yellow No. 7 at a dose of 250 mg/kg bw/day was associated with liver and kidney changes. At 80 mg/kg bw/day the only findings attributable to the test article were orange fur and tail staining, coloration of the urine, and a bilateral coloration of the fundus of the eye. The study authors concluded that the NOAEL was 80 mg/kg bw/day.
The basophilia suggests regeneration of damaged cells and is common if tubular nephrosis is reported. The toxicological importance of the observations in the eye at 80 mg/kg bw/day are not clear and therefore the NOAEL should be 25 mg/kg bw/day.

Ref. : 5

2.3.5. Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Sub-chronic oral toxicity

No data

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 and corrosivity

2.4.1. Irritation (skin)

Guideline	:	Journal Officiel de la République Française, 21 February 1982
Species/strain	:	New Zealand Albino rabbits
Group size	:	6 males
Test substance	:	HC Yellow No. 7 at 0.5 % in propylene glycol
Batch No.	:	DG 3
Purity	:	/
Dose	:	0.5 ml
GLP	:	In compliance

The substance (0.5 ml) was applied to a 6.25 cm^2 area of intact and abraded skin of 6 male rabbits. Occlusive patches were applied and left in place for a 24-hour period. Remaining test substance was removed by swabbing with cotton wool swabs. The skin was examined for erythema, eschar formation, and oedema at 24 and 72 hours after removal of the patches.

Results
No signs of irritation were noted on the skin. Slight staining of the skin was noted in all animals. The primary irritation index was 0.0 and the substance was non-irritant to rabbit skin.

Ref. : 3

2.4.2. Irritation (mucous membranes)

Guideline	:	Journal Officiel de la République Française, 1971 and 1973
Species/strain	:	New Zealand Albino rabbits
Group size	:	6 males
Test substance	:	HC Yellow No. 7 dissolved at 0.5 % in propylene glycol
Batch No.	:	DG 3
Purity	:	/
Dose	:	0.1 ml
GLP	:	In compliance

0.1 ml of 0.5% (w/v) of the test substance was applied once to one eye of each animal without rinsing. The other eye served as control. Ocular reactions were recorded at 24, 48, and 72 hours, and 4 and 7 days after instillation.

Results
No reactions were observed in the cornea or iris of any animals. Irritation of the conjunctiva was seen in 5/6 animals at 24 hours after instillation only. According to the defined criteria of this study, the substance was classified as minimally irritant to the rabbit eye.

Ref. : 2

2.5. Sensitisation

Magnusson and Kligman Maximisation Test

Guideline : OECD 406 (1981)

Species/strain	:	Hartley/Dunkin albino guinea pigs	
Group size	:	20 test + 10 control, females	
Test substance	:	HC Yellow No. 7 in Alembicol D®	
Batch No.	:	DG 3	
Purity	:	/	
Concentration	:	intradermal induction :	0.1 ml 50% Freund's complete adjuvant (FCA)
			0.1 ml 0.5% (w/v) test substance
			0.1 ml 0.5% (w/v) test substance/FCA
		topical induction:	0.5 ml 0.5% test substance in Alembicol D®
		challenge:	0.2 ml 5% and 2.5% test substance for 24 hours, occluded
			0.2 ml 2.5% test substance for 24 hours, occluded
GLP	:	In compliance	

A preliminary intradermal study indicated that 5% (w/v) test substance could be used without provoking an irritant response. Induction commenced with three pairs of intradermal injections of FCA, test substance (0.5%), and a mixture of the two. The induction process was completed on day 8 with a single topical application of 0.5 ml of test substance (5%) under occlusive patch to the shoulder region for 48 hours. An interval of two weeks was allowed after induction and then the animals were challenged by a single topical application of the test substance (5% and 2.5%) under occlusive patch on the left flank for 24 hours. Appropriate controls were treated with vehicle at all stages and the test substance-induced animals received vehicle alone on the opposite flank.
The skin was examined 24 hours after administration of the intradermal injection and again after removal of the topical patches for signs of irritation. The skin was examined 24, 48, and 72 hours after removal of the challenge patches.

Results
The substance provoked irritation in both induction periods. All test animals showed evidence of erythema and oedema after the challenge. The test substance produced evidence of delayed contact hypersensitivity to guinea pig skin.

Ref. : 4.1

Epicutaneous Maximisation Test

Guideline	:	Journal Officiel de la République Française, norm No. T03-300	
Species/strain	:	Dunkin-Hartley guinea pigs	
Group size	:	10 males + 10 females	
Test substance	:	IMEXINE AA in PEG 300	
Batch No.	:	DG 3	
Purity	:	/	
Concentration	:	induction:	0.1 ml 50% Freund's complete adjuvant (FCA)
			0.5 ml 5% test substance for 7 applications (days 1-15)
		challenge:	0.5 ml 5% test substance for 48 hours, occluded
GLP	:	In compliance	

A preliminary intradermal study indicated that 5 % (w/v) test substance could be used without provoking an irritant response. Induction commenced with an intradermal injection of FCA. The test substance (0.5 ml at 5 %) was applied under occluded patch for 48 hours, three times a

week, every other day, for two weeks, and once at the start of week 3, immediately behind the right shoulder. After a 12-day rest period, the animals were challenged by a single topical application of the test substance (0.5 ml at 5 %) under occlusive patch on the left flank for 48 hours. The skin was examined for erythema and oedema, 1, 6, 24, and 48 hours after removal of the challenge patches.

Results
One male animal died during the test period and was replaced. The cause of death was not established. Staining of the skin prevented assessment of erythema. No cutaneous reactions were observed. The test is considered inadequate.

Ref. : 4.2

2.6. Teratogenicity

Guideline	:	OECD 414 (1981)
Species/strain	:	Sprague Dawley. Crl: CD (SD) BR
Group size	:	25 females (mated)
Test substance	:	IMEXINE AA suspended in 0.5% aqueous carboxymethylcellulose
Batch No.	:	Op. 26
Purity	:	99.9 %
Dose levels	:	0, 25, 80, and 240 mg/kg bw/day
Treatment period	:	Days 6-15 of pregnancy, inclusive
GLP	:	In compliance

Groups of 25 female rats were dosed with the test substance by gavage on days 6 to 15 after mating. The control group received the vehicle alone. The dams were observed daily for clinical signs and mortality. Body weight and food consumption was recorded on days 0, 6, 9, 12, 15, and 20. The dams were sacrificed on day 20 of pregnancy, and examined for number of corpora lutea, number and distribution of live and dead foetuses, of early or late resorptions and of implantation sites, and for macroscopic observations. The foetuses were examined for body weight, sex and macroscopic external observations, and for skeletal and visceral abnormalities (half for each endpoint).

Results
One dam treated with the high dose had piloerection, paleness of the extremities, ataxia and yellowish ocular discharge on day 9 of gestation, and was sacrificed as it was moribund. No other deaths or clinical signs of toxicity were reported and there were no abortions at any dose level. Orange coloured urine was noted for all treated dams from day 1 to 4 days after the beginning of treatment until one day after treatment stopped. The body weight gain of the females treated with 25 mg/kg bw/day was comparable to controls. In the other groups, the body weight gain was lower than the controls (62 g, 52 g, and 29 g, for control, 80, and 240 mg/kg/day, respectively). Dams of the highest dose group lost weight during the first three days of treatment. Food consumption was about 25% lower than controls in rats treated with 240 mg/kg bw/day, and returned to control values after treatment was stopped. Food consumption for other groups was comparable to controls.
No abnormalities were observed in any of the dams at the scheduled autopsy. The female that was sacrificed on day 9 had yellowish coloration of the tissues and enlarged mandibular ganglions. The mean numbers of corpora lutea, implantation sites, post-implantation loss, live foetuses, and foetal body weights were similar for control and groups treated with 80 and 240 mg/kg bw/day. In the 25 mg/kg bw/day group, there was a reduction in the number of corpora

lutea and, as a consequence, a reduction in the number of live foetuses. The incidence of skeletal abnormalities related to delayed ossification was significantly greater at 240 mg/kg bw/day than in controls. A small number of foetal malformations were observed, which were within the normal range, and treated groups did not differ significantly from control.
There was slight maternal toxicity at 80 mg/kg bw/day, and moderate maternal toxicity, with subsequent delayed foetal skeletal development, at 240 mg/kg bw/day, but no evidence of embryo-toxicity or teratogenicity. The NOAEL for maternal toxicity was considered to be 25 mg/kg bw/day.

Ref. : 11

2.7. Toxicokinetics (including Percutaneous Absorption)

2.7.1. Percutaneous Absorption *in vitro*

Study 1

Guideline	:	/
Tissue	:	Human abdominal epidermis
Method	:	Franz diffusion cell (static)
Test substance	:	IMEXINE AA, 0.12% in formulation
Batch no	:	Op 26
Purity	:	/
Dose levels	:	Circa 40mg formulation in the presence/absence of hair
Replicate cells	:	8
GLP	:	Not in compliance

The skin penetration of HC Yellow No. 7 was evaluated in a static Franz diffusion cell using human epidermis prepared by heat-separation of previously frozen abdominal skin. The test substance was prepared at a concentration of 0.12% in a formulation. Approximately 40 mg of the mixture was applied to $2cm^2$ of epidermal membrane for 30 minutes, with and without addition of finely chopped bleached hair and then excess washed off with distilled water and 2% sodium lauryl sulphate solution and dried. Four hours later the levels of substance were measured in the receptor fluid (physiological saline) using HPLC. Integrity of the epidermal membrane was checked by microscopy before the study and with Chinese ink at the end of the study.

Results
The quantity of test substance penetrating through the epidermis to the receptor fluid corresponded to 0.094% of applied dose in the presence of hair and 0.124% of applied dose in the absence of hair.
This study did not include determination of recovery of the test substance. Physiological saline was used as the receptor fluid, which may not be adequate for a relatively lipophilic substance, and insufficient time was allowed for penetration from the epidermal membrane into the receptor fluid. The study is considered inadequate.

Ref. : 12

Study 2

Guideline : Predates OECD 428 (2000)

Test substance	:	IMEXINE AA
Batch No.	:	05033175
Purity	:	/
Tissue	:	Human abdominal dermatomed skin (kept at -20 °C)
Skin integrity	:	TEWL measurement
Method	:	Static diffusion cell 2 cm^2; receptor compartment 3 ml
Receptor fluid	:	Instamed® PBS buffer w/o calcium and magnesium salts; 9.55 g/l, containing 0.25% of Tween 80
Formulation tested	:	Typical commercial formula
Dose of formulation applied	:	20 mg/cm^2
Concentration of ingredient	:	0.11% (amount applied, 21.7 ± 0.6 $\mu g/cm^2$)
Replicate cells	:	4 skin donors, 2 cells/donor, 8 cells mounted, and interpreted
Duration of contact	:	30 minutes
Duration of diffusion	:	24 hours
Analytical method	:	HPLC with visible detection
Validation	:	Limit of detection and limit of quantitation measured in the receptor fluid and in the extraction solvent of tissue samples
Solubility in receptor fluid	:	Verified at 32 °C, > 15.9 µg/ml
Stability of ingredient	:	No information
GLP	:	In compliance

The skin penetration of HC Yellow No. 7 was evaluated in a static Franz diffusion cell system. Human abdominal skin previously frozen was dermatomed to a constant thickness (593 ± 148 µm). The integrity of the skin was evaluated by the measurement of the TEWL; the skin surface temperature was monitored (32.1 ± 0.4 °C). The solubility of the substance in the receptor fluid (PBS buffer with 0.25% of Tween 80 as a solubilizer) was checked in the range of the concentrations used. The test substance was prepared at a concentration of 0.11% in a "commercial type" formulation. Approximately 20 mg/cm^2 of the formulation (exactly measured) were applied to 2 cm^2 for 30 minutes. The excess from the skin surface was rinsed first with water, followed by a wash with 2% sodium lauryl sulphate aqueous solution, again rinsed with water, and finally dried with a cotton swab. 24 hours after the application, the substance was measured using HPLC in the receptor fluid, in the horny layer collected by tape stripping (2 to 17 strips), in the epidermis and dermis altogether, and in the remaining skin outside the application area. After assay of HC Yellow No. 7 in the washing material (skin excess), the mass balance of the study was calculated (105.09 ± 3.38 % of the applied dose).

Results

Most of the hair dye applied was recovered at the skin surface in the washing liquids (104.95 ± 0.37 %). The quantity of test substance penetrating through the skin to the receptor fluid was 0.01 ± 0.02 % of the applied dose (0.003 ± 0.006 $\mu g/cm^2$). The amount recovered in the horny layer was 0.09 ± 0.13 % (0.023 ± 0.028 $\mu g/cm^2$): it was not considered to be percutaneously absorbed. The epidermis and the dermis content was 0.04 ± 0.02 % of the applied dose (0.008 ± 0.004 $\mu g/cm^2$). The amount found in the horny layer was higher than that in the dermis and epidermis, indicating a storage phenomenon of the hair dye in the *stratum corneum*.

The absorbed amounts of HC Yellow No. 7 (epidermis + dermis + receptor fluid) represents 0.05 ± 0.003 % of the applied dose (0.011 ± 0.008 $\mu g/cm^2$) at the end of 24 hours of diffusion after a contact with the skin of 30 minutes.

Ref. : 14

2.8. Mutagenicity/Genotoxicity

2.8.1. Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline	:	OECD 471 (1983)
Species/strain	:	*S. typhimurium,* TA98, TA100, TA1535, TA1537, *E. coli* WP2 uvr A
Replicates	:	Triplicate plates, 2 independent tests
Test substance	:	IMEXINE AA dissolved in DMSO
Batch No.	:	Op. T 68
Purity	:	99.9 %
Concentrations	:	156.25-2500 μg/plate, with and without metabolic activation
GLP	:	In compliance

HC Yellow No. 7 has been investigated for gene mutation in *S. typhimurium* and *E. coli* using the plate incorporation method. Liver S9 fraction from Aroclor 1254 treated rats was used as the exogenous metabolic activation system. Negative and positive controls were in accordance with the OECD guideline. The concentration range 156.25-2500 μg/plate was selected on the basis of a preliminary toxicity indicating that 2500 μg/plate was cytotoxic and at the limit of solubility.

Results
The substance induced a concentration-related increase in numbers of revertants in TA1537 and TA98 with metabolic activation. There were no significant increases with other strains or in the absence of S9. The negative and positive control agents gave the expected results. The compound is considered mutagenic in this system.

Ref. : 6

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

Guideline	:	OECD 476 (1984)
Cells	:	L5178Y ($TK^{+/-}$) mouse lymphoma cells
Replicates	:	2 independent tests
Test substance	:	IMEXINE AA in DMSO solution
Batch No.	:	Op. T 68
Purity	:	99.9 %
Concentrations	:	37.5-600 μg/ml, with and without metabolic activation
GLP	:	In compliance

HC Yellow No. 7 has been investigated for gene mutation at the TK locus in L5178Y ($TK^{+/-}$) mouse lymphoma cells. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. The concentration range 37.5-600 μg/ml was selected on the basis of a preliminary toxicity indicating that concentrations of 1000 and 5000 μg/ml were cytotoxic. Negative and positive controls were in accordance with the OECD guideline.

Results
In both experiments the substance induced a concentration-related increase in mutation frequency in the absence of S9. In the presence of S9, an increase was seen only at 300 μg/ml,

which was associated with severe toxicity. No cells survived at higher concentrations. The negative and positive control agents gave the expected results. The compound is considered mutagenic in this system.

Ref. : 7

***In vitro* Mammalian Chromosomal Aberration Test**

Guideline	:	OECD 473 (1983)
Species/strain	:	Chinese Hamster Ovary cells
Replicates	:	Duplicate cultures, 2 independent tests
Test substance	:	IMEXINE AA in DMSO
Batch No.	:	T 62/2
Purity	:	/
Concentrations	:	10, 50, and 100 μg/ml without metabolic activation; 20, 100, and 200 μg/ml in Experiment 1 with metabolic activation; 10, 50, and 100 μg/ml in Experiment 2 with metabolic activation
GLP	:	In compliance

HC Yellow No. 7 has been investigated for induction of chromosomal aberrations in Chinese Hamster Ovary (CHO) cells with continuous exposure and 24- and 48-hour harvest times. Liver S9 fraction from rats pre-treated with β-naphthoflavone and sodium phenobarbitone was used as the exogenous metabolic activation system. The test concentrations were selected so that the highest concentration decreased the mitotic index by more than 50% of control. Negative and positive controls were in accordance with the OECD guideline. The treatment time (continuous exposure for 24 and 48 hours) was not in accordance to OECD guideline: a mitotic delay or an extreme toxicity may have been produced, thus influencing the results obtained.

Results
The study reported negative results at all conditions in the first experiment. The second experiment produced equivocal results. Especially in the first experiment, the cells of the untreated control did not show the "normal" occurrence of baseline structural or numerical chromosome aberration. The cell line used might have been resistant and not appropriate for genotoxicity testing. Moreover, no historical control data were presented that could allow a more accurate interpretation of the results. The study is unsuitable for genotoxicity evaluation.

Ref. : 8

2.8.2. Mutagenicity/Genotoxicity *in vivo*

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

Guideline	:	OECD 475, draft (1991)
Species/strain	:	Wistar rat, HanIbm: WIST (SPF) strain
Group size	:	4 males
Test substance	:	IMEXINE AA in PEG 300
Batch No.	:	Op. T 68
Purity	:	/
Dose levels	:	25 and 250 mg/kg, by gavage
Sacrifice times	:	16 hours, all dose groups; 2 hours, high dose group
GLP	:	In compliance

HC Yellow No. 7 has been investigated for induction of unscheduled DNA synthesis (UDS) in rat hepatocytes *in vitro* following *in vivo* dosing. A preliminary toxicity study indicated that 250 mg/kg bw was close to the MTD and therefore this was used as the highest dose. Negative and positive controls were in accordance with the OECD guideline.
Animals were sacrificed after 16 hours and for an additional high dose group after 2 hours. Hepatocytes were isolated and at least 3 cultures were established per animal. The hepatocytes were subsequently treated with ^{3}H-thymidine *in vitro* for 4 hours. Incorporation of radio-label was assessed using auto-radiography.

Results
One animal died within 16 hours of dosing at 250 mg/kg; the cause of death was not established. There were no differences in the viability of hepatocytes isolated from rats of different dose groups. The results met all the pre-defined criteria for a negative response and therefore the test substance was not found to induce UDS. The positive control agent gave the expected results.

Ref. : 9

Mammalian Erythrocyte Micronucleus Test

Guideline	:	/
Species/strain	:	Mouse, Swiss Albino strain
Group size	:	10 males
Test substance	:	HC Yellow No. 7 in 20% DMSO-glycerol (1:4)
Batch No.	:	JJXV 14
Purity	:	/
Dose levels	:	0, 75, 125, and 175 mg/kg bw/day, twice i.p.
Sacrifice times	:	6 hours
GLP	:	Not in compliance

HC Yellow No. 7 has been investigated for induction of micronuclei in the bone marrow cells of mice. The substance was administered by intraperitoneal injection twice at a 24-hour interval, and groups of animals were sacrificed at 6 hours after the last administration for harvest of bone marrow cells. No positive controls were used.

Results
No positive control agent was used and therefore the sensitivity of the study could not be demonstrated. The study is considered unsuitable for genotoxicity evaluation.
The study was conducted in 1983 and the report prepared in 1992. The reason for this delay is not explained.

Ref. : 10

2.9. Carcinogenicity

No data

2.10. Special investigations

No data

2.11. Safety evaluation

NOT APPLICABLE

2.12. Conclusions

Chromatographic purity of the compound has not been reported. Chemical purity was not stated in many toxicity study reports. Trace unknowns were detected, whose health hazard properties have not been clarified. No adequate experimental data on stability were provided.

HC Yellow No. 7 was "minimally irritant" to the rabbit eye and non-irritant to rabbit skin. It has shown evidence of sensitisation at a concentration of 2.5%.

A 4-week rat oral study gave evidence of liver and kidney damage at 250 mg/kg bw/day and staining of the fundus of the eye at 80 mg/kg bw/day. The NOAEL was 25 mg/kg bw/day. This is consistent with results of a teratogenicity study in which slight maternal toxicity was seen at 80 mg/kg bw/day, and moderate maternal toxicity, with subsequent delayed foetal skeletal development, at 240 mg/kg bw/day. The NOAEL for maternal toxicity was considered to be 25 mg/kg bw/day.

Percutaneous penetration : almost all of the hair dye applied was recovered at the skin surface in the washing liquids. The absorbed amounts of HC Yellow No. 7 (epidermis + dermis + receptor fluid) represents 0.05 ± 0.003 % of the applied dose (0.011 ± 0.008 $\mu g/cm^2$) at the end of 24 hours of diffusion after a contact with the skin of 30 minutes.

The substance induced gene mutations in bacteria and mammalian cells. It was negative in an *in vivo* UDS study. The *in vitro* chromosome aberration study and the *in vivo* micronucleus test are considered inadequate. Therefore, no conclusions can be drawn for this compound as to its genotoxicity/mutagenicity potential.

2.13. References

1. Huntingdon Research Centre, UK. Report No. 84526 D/LRL/ 21/AC (July 1984)
2. Huntingdon Research Centre, UK. Report No. 84437D/LRL 20/SE (July 1984)
3. Huntingdon Research Centre, UK. Report No. 8431/D/LRL 19/SE (May 1984)
4.1. Huntingdon Research Centre, UK. Report No. 8534/D/LRL 24/SS (February 1985)
4.2. Hazleton IFT, France. Report No. 508435 (August 1985)
5. CIT, France. Study No. 6447 TSR (December 1991)
6. CIT, France. Report No. 11651 MMJ (August 1994)
7. CIT, France. Report 11652 MLY (November 1994)
8. Toxicol Labs, UK. Study No. M/CCA/38503 (March 1994)
9. Cytotest Cell Research GmbH, Germany. Report No. 491700 (February 1995)
10. Department des "Controles Biologiques", France. Study: August 1983; Report No. 08/83 (June 1992)
11. CIT, France. Report 7035 RSR (August 1991)
12. L'Oreal, France. Refs: 90/01/036 and 90/01/037 (September 1990)
13. CIT, France. Imexine AA, micronucleus test by oral route in mice. Study n° 13644 MAS, 18 June 1996

14. L'Oréal Recherche, France. In vitro percutaneous absorption of Imexine AA (Colipa n° B80). Study n° 16080; 18/12/2000

3. Opinion of the SCCNFP

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

Before any further consideration, the following information is required :

* proper analytical and physico-chemical data, e.g. characterisation of the purity/impurities of all the batches used, related health hazards of impurities, experimental data on stability, sensitivity to light and moisture.

* data on 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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