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

CONCERNING

HC ORANGE NO. 3

COLIPA n° B68

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 Orange No. 3 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 Orange No. 3 (INCI name)

2.1.2. Chemical names

Chemical name : 1-(2,3-Dihydroxypropyloxy)-3-nitro-4-(2-hydroxyethylamino)-benzene
CAS name : 1,2-Propanediol, 3-[4-[(2-hydroxyethyl)amino]-3-nitrophenoxy]-
Synonyms : 3-[4-(2-Hydroxy-ethylamino)-3-nitro-phenoxy]-propane-1,2-diol

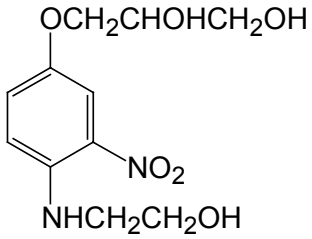
2.1.3. Trade names and abbreviations

Trade name : IMEXINE® FAC (Chimex)
COLIPA No. : B-68

2.1.4. CAS No. / EINECS No.

CAS No. : 81612-54-6
EINECS No. : /

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. formula : $C_{11}H_{16}N_20_6$
Mol. weight : 272.26

2.1.7. Purity, composition, and substance codes

All analytical data relate to Batch Op. 10

Purity

titre as determined by spectrophotometry	:	96%
water content (Karl Fischer)	:	0.7% (w/w)
ash content	:	0.94% (w/w)

Potential impurities and reaction intermediates

4-(2-hydroxy-ethylamino)-3-nitro-phenol (Impurity A) : 0.66% (w/w)

At least three other impurities were detected by HPTLC and HPLC (possibly combined with MS): an isomeric form of HC Orange No. 3 (Impurity B), not quantified for lack of standard, and two trace unknowns.

Solvent residues

ethanol	:	< 100 ppm
n-propanol	:	0.17%
acetone	:	< 100 ppm

Other

heavy metals	:	< 10 ppm
chloride ions	:	0.26 mEq/g

2.1.8. Physical properties

Appearance	:	Bright red powder; almost odourless
Melting point	:	106 °C, with decomposition
Boiling point	:	/
Density	:	0.4 g/cm^3
Rel. vap. dens.	:	/
Vapour press.	:	/
Log P_{OW}	:	0.2 (calculated); 0.0 (measured at pH 7.2)
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	:	1 %
Ethanol, 95%	:	1 %,
Dimethylformamide	:	10 %.
Receptor fluid*	:	≥ 100 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 dye was not reported.

* Purity of the chemical reported for one batch only (Batch Op. 10) : it would be advisable to have an statement of the range of impurities that be may be present, based on the analysis of more than one batch.

* Two low-level impurities and two 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.

* Log P_{OW} experimental protocol not specified (however, log P_{OW} estimates reported appear to be coherent with each other and with the chemical functions of the compound).

* No experimental data on stability provided; suggestion of chemical sensitivity to light and moisture.

* HC Orange 3 is a secondary alkanolamine, and thus, it is prone to nitrosation. The contents of nitrosamine in the dye as well as in the hair dye formulations have not been reported

2.2. Function and uses

HC Orange No. 3 will be incorporated into semi-permanent hair dyes at a maximum concentration of 0.5%. 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 (1982)
Species/strain	:	Sprague-Dawley rats. Crl: CD(SD) BR
Group size	:	5 males + 5 females
Test substance	:	IMEXINE FAC suspended in 0.5% aqueous carboxymethylcellulose
Batch No.	:	Op. 10
Purity	:	/
Dose	:	1000 mg/kg bw
Observ. period	:	14 days
GLP	:	In compliance

The dose selected was 1000 mg/kg bw. Groups of 5 males and 4 females received a single dose of test substance by gastric gavage. The animals were observed daily after dosing. Body weights were recorded on days 1, 8, and 15 of the study. Macroscopic examination of main organs was performed at autopsy. No histological examinations were performed.

Results
There were no clinical signs of toxicity or mortalities. Body weight gain was considered normal for the age and strain of rat. No abnormalities were recorded at autopsy. The LD_{50} was in excess of 1000 mg/kg bw/day.

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

No data

2.3.5. Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Sub-chronic oral toxicity

Guideline	:	OECD 408 (1981)
Species/strain	:	Sprague Dawley rats. Crl: CD(SD) BR
Group size	:	10 males + 10 females
Test substance	:	IMEXINE FAC suspended in 0.5% aqueous carboxymethylcellulose
Batch No.	:	Op. 10
Purity	:	/
Dose	:	0, 100, 300, and 1000 mg/kg bw/day
Exposure period	:	13 weeks (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, 100, 300, and 1000 mg/kg bw/day, 7 days a week for 13 weeks. The dosing solutions were analysed during weeks 1, 4, 8, and 13 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 weeks 4 and 13, urine was collected overnight for urinalysis, and blood was sampled from the lateral tail vein 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 before the start of the study and at the end of the treatment period on control and high dose animals.

Results
No mortalities occurred. Orange staining of the fur and tail attributable to the compound was reported in all treated animals. Other clinical signs, such as hair-loss and scabbing occurred in controls as well as test animals and were not considered to be treatment-related. Bodyweight gains were higher in some treated groups (males at 100 and 1000 mg/kg/day, females at 300 mg/kg/day) than in control, but not in a dose-related fashion. Food consumption was

comparable for all dose groups. All treated animals (high dose) had a slight orange reflective colour from the background sclera on ophthalmological examination of the retina. The authors considered this to be attributable to the properties of the test substance. There were no other treatment-related findings in the ophthalmoscopy.
Several small but statistically significant changes were seen in the red blood cell parameters at both week 4 and week 13. They did not show consistent time and dose-related trends and were within the normal range and therefore not considered to be treatment-related.
There was a dose-related increase in blood glucose in male animals in both week 4 and week 13, which was statistically significant at all dose levels in week 13. The study authors considered that this was not of toxicological importance because the values were within the normal reference range.
Alanine aminotransferase levels were elevated in the week 13 sample of males dosed at 1000 mg/kg bw/day. This increase was accompanied by an increase in AST, which was not, however, statistically different from control. Other small significant changes were reported but were within the normal range and not dose-related, and thus considered to be unrelated to treatment.
Discoloration of the urine precluded most urinary measurements for animals treated at 300 and 1000 mg/kg bw/day. No changes were reported for urine samples which could be analysed.
Liver weight showed a dose-related increase in females, for which the absolute weights was significantly different from control at both 300 and 1000 mg/kg bw/day (113% and 121%, respectively) whereas the relative weight was only significant at the high dose (115%). Liver weight also appeared elevated for the high dose males, but were not significantly greater than control (absolute weight 121%, relative weight 116%). A similar pattern was seen for increases in spleen and kidney weights, for which the absolute weights were significant in females at 300 and 1000 mg/kg bw/day (spleen absolute weights: 120% and 127%; spleen relative weights: 114% and 118%; kidney absolute weights: 111% and 115%; kidney relative weights: 103% and 109%, respectively). The authors considered the spleen and kidney findings not to be of toxicological relevance because the weights were within the normal range.
The only notable macroscopic findings related to the staining properties of the hair dye. The small number of histological findings recorded were reported to be within the normal range for this strain of rat. There was no evidence of toxicity in any organ, including the liver, spleen and kidney.
Based upon the observations of elevated alanine aminotransferase and liver weight in males at 1000 mg/kg bw/day, the authors concluded that the NOAEL was 300 mg/kg bw/day. The SCCNFP concluded that the NOAEL should be 100 mg/kg bw/day on the basis of the observed changes in organ weights.
Fur and tail staining, observed in all treated animals during the treatment period as well as discoloration of the urine and staining of the urinary bladder, are indicative of absorption and distribution of the substance.
The subchronic toxicity study lacks a recovery group which could give an answer to the question if the staining of the eyes observed in the high dose group is persistent. In addition, no data are available for the dose groups 100 and 300 mg/kg bw/day. However, within the irritation study, the ocular reactions were recorded at 24, 48, and 72 hours after instillation. The red staining was only recorded up to 24 h. This may point to a reversible staining effect in the eyes.

Ref. : 5

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No data

2.4. Irritation and corrosivity

2.4.1. Irritation (skin)

Guideline	:	OECD 404 (1987)
Species/strain	:	New Zealand Albino rabbits
Group size	:	3 males
Test substance	:	IMEXINE FAC
Batch No.	:	Op. 10
Purity	:	/
Dose	:	0.5 g
GLP	:	In compliance

The substance (0.5 g) was applied to a 6.25 cm^2 area of intact skin of 3 male rabbits. Semi-occlusive patches were applied and left in place for a 4-hour period. Remaining test substance was removed by swabbing with cotton wool swabs soaked in warm water. The skin was examined for erythema, eschar formation, and oedema at 1, 24, 48, and 72 hours after removal of the patches. A primary irritation index was calculated from the mean scores at the sites and at each time point according to Draize (modified).

Results
Orange staining of the fur around the treatment sites was reported for all animals, but no signs of erythema or oedema were noted. The substance was non-irritant to the skin of the albino rabbit.

Ref. : 3

2.4.2. Irritation (mucous membranes)

Guideline	:	OECD 405 (1987)
Species/strain	:	New Zealand White rabbits
Group size	:	3 females
Test substance	:	IMEXINE FAC
Batch No.	:	Op. 10
Purity	:	/
Dose	:	0.1 g neat substance
GLP	:	In compliance

0.1 g of the neat substance was applied once to the right eye of each animal without rinsing. The left eye served as control. Ocular reactions were recorded at 24, 48, and 72 hours after instillation.

Results
Some red staining was seen in one rabbit up to 24 hours after dosing. No evidence of eye irritation was observed and the substance was classified as non-irritant to the rabbit eye. Persistence of the stain in the eye for 24 hours could be analogous to the ophthamological observation reported in the 13-week study.

Ref. : 2

2.5. Sensitisation

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 FAC in propylene glycol solution
Batch No.	:	DG 1
Purity	:	/
Concentration	:	Induction: 0.1-ml 50% Freund's complete adjuvant (FCA) 0.5-ml 5% test substance for 7 applications Challenge: 0.5-ml 5% test substance for 48 hours, occluded (day 28)
GLP	:	Not in compliance

A preliminary intradermal study indicated that 5% test substance could be used without provoking an irritant response. Induction commenced with an intradermal injection (0.1 ml) of FCA. The test substance (0.5 ml at 5%) was applied under occlusive patch for 48 hours, three times a week, every other day, for two weeks, and once at the start of week 3 to the right flank above the injection site. After a 12 day rest period the animals were challenged by a single topical application of 0.5-ml test substance (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
Five animals died during the test period and were replaced. The cause of death was not established. Staining of the skin prevented proper assessment of erythema. The test is considered inadequate.

Ref. : 4

2.6. Teratogenicity

Guideline	:	OECD 414 (1981)
Species/strain	:	Sprague Dawley rats. Crl: CD(SD)BR
Group size	:	24 females (mated)
Test substance	:	IMEXINE FAC suspended in 0.5% aqueous carboxymethyl cellulose
Batch No.	:	Op. 10
Purity	:	/
Dose levels	:	0, 300, and 1000 mg/kg bw/day
Treatment period	:	Days 6-15 of pregnancy, inclusive
GLP	:	In compliance

Groups of 24 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 weights were monitored on days 0, 6-15, and 20. 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 end point).

Results
No deaths or abortions occurred. Clinical signs were limited to hairloss and scabbing throughout all dose groups, including control, and orange staining of the fur, tail, and urine in all treated groups. Food consumption and body weight gain were comparable for all dose groups. At autopsy, no abnormalities were observed except for those related to staining by the dye. The mean numbers of corpora lutea, implantation sites, post-implantation loss, live foetuses, and foetal body weights were similar for control and treated groups.
A total of 2, 1, and 2 foetuses exhibited major skeletal and external/visceral abnormalities at 0, 300, and 1000 mg/kg bw/day, respectively. Because the malformations were known to occur in this strain of rat and were not dose-related, they were not considered to be of toxicological importance. The incidence of minor abnormalities was low, within the normal range, and was not significantly different between dose groups.
The test substance did not elicit maternal or developmental toxicity at doses up to 1000 mg/kg bw/day.

Ref. : 10

2.7. Toxicokinetics (including Percutaneous Absorption)

2.7.1. Percutaneous Absorption *in vitro*

Study 1

Guideline	:	/
Tissue	:	Human breast and abdominal epidermis
Method	:	Franz diffusion cell (static)
Test substance	:	IMEXINE FAC , 0.36% in formulation mix pH 9.5
Batch No.	:	Op.10
Purity	:	/
Dose levels	:	Circa 40 mg formulation in the presence/absence of 10 mg hair
Replicate cells	:	17 without hair, 18 with hair
GLP	:	Not in compliance

The skin penetration of HC Orange No. 3 was evaluated in a static Franz diffusion cell using human epidermis prepared by heat-separation of previously frozen breast or abdominal skin. The test substance was prepared at a concentration of 0.36% 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 0.027% of applied dose in the presence of hair and 0.236% of applied dose in the absence of hair.
This study was conducted with a concentration less than the intended use level, it did not include determination of recovery of the test substance and insufficient time was allowed for penetration from the epidermal membrane into the receptor fluid.
The study is considered inadequate.

Ref. : 11

Study 2

Guideline	:	OECD 428, draft (approved by WNT, May 2002)
Tissue	:	Human dermatomed skin samples
Method	:	Franz diffusion cells
Test substance	:	HC Orange No. 3, 0.502% in formulation
Batch No	:	T10
Purity	:	96 %
Dose levels	:	20 mg/cm^2 of formulation; 0.1 mg/cm^2 of HC Orange No. 3
Receptor fluid	:	Instamed® PBS buffer w/o Ca^{2+}, Mg^{2+} 9.55 g/l, containing 0.25% of Tween 80
Replicate cells	:	8 cells
Analyt. method	:	HPLC (visible detection, 482 nm); quantitation limit: 0.002-0.1 µg/ml (receptor fluid)
Stability	:	No loss of material was observed after 1 month at room temperature
GLP	:	In compliance

The skin penetration of HC Orange No. 3 was evaluated in a static Franz diffusion cell system using human dermatomed skin samples. The integrity of the skin was checked by TEWL. According to the solubility parameters of the chemical and its log P_{OW}, an appropriate saline buffer was used as receptor fluid. The solubility of the substance in the receptor fluid was ≥ 100 µg/ml.
Twenty mg/cm^2 of a hair dye formulation containing 0.502% (w/w) of HC Orange No. 3 were applied on the skin surface for 30 min. Then, the skin surface excess was washed off with a 2% sodium dodecyl sulfate solution, rinsed with water and finally dried. Twenty-four hours after application, the content of the substance was determined by HPLC in the following compartments: skin excess, SC, epidermis + dermis, and receptor fluid.

Results
Under the experimental conditions described, a total recovery of 103.32% was obtained. Most of the hair dye applied on the skin surface was removed with the washing procedure (99.24% of the applied dose). The content of the test substance detected in the SC was 0.148 µg/cm^2 (0.15% of the applied dose). The amount of the substance in the epidermis and dermis accounted for 0.049 µg/cm^2 (0.05% of the applied dose). Almost no diffusion of HC Orange No. 3 in the receptor fluid was observed (0.003 µg/cm^2).
The absorbed amount of the chemical accounted for by the epidermis + dermis + receptor fluid compartments was 0.053 µg/cm^2 (0.05% of the applied dose).

Ref. : 13

2.8. Mutagenicity/Genotoxicity

2.8.1. Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline	:	/
Species/strain	:	*S. typhimurium*, TA98, TA100, TA1535, TA1537, TA1538, *E. coli* WP2 uvr A
Replicates	:	Triplicate plates, 2 independent tests
Test substance	:	IMEXINE FAC dissolved in DMSO
Batch No.	:	Op. 10
Purity	:	/
Concentrations	:	8-5000 μg/plate with and without metabolic activation
GLP	:	In compliance

HC Orange No. 3 has been investigated for gene mutation in *S. typhimurium* and *E. coli* using the plate incorporation method. Liver S9 fraction from rats pretreated with β-naphthoflavone and sodium phenobarbitone was used as the exogenous metabolic activation system. Negative and positive controls were adequate. A preliminary toxicity study showed no toxicity up to 5000 μg/plate, which was therefore selected as the maximum concentration according to guidelines.

Results
The test substance induced a concentration-related increase in the number of revertants in TA100 with metabolic activation. No increases were seen without S9, or in the other tester strains. The negative and positive control agents gave the expected results. The test was acceptable for evaluation and the substance was considered to be mutagenic.

Ref. : 6

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

Guideline	:	/
Species/strain	:	Mouse lymphoma L5178Y $TK^{+/-}$ cells
Replicates	:	2 independent tests
Test substance	:	IMEXINE FAC in dimethylformamide solution
Batch No.	:	Op. 10
Purity	:	/
Concentrations	:	200, 400, 1000, and 2000 μg/ml with and without metabolic activation
Exposure	:	3 hours
GLP	:	In compliance

HC Orange No. 3 has been investigated for induction of cell mutations at the TK locus in mouse lymphoma L5178Y $TK^{+/-}$ cells. Liver S9 fraction from rats pretreated with β-naphthoflavone/phenobarbitone was used as the exogenous metabolic activation system. A preliminary toxicity study established that toxicity started to occur in the region of 1000 μg/ml, with and without metabolic activation, and test concentrations were therefore set at 200, 400, 1000, and 2000 μg/ml.

Results
There were no consistent increases in mutation frequency. A statistically significant increase was seen only at 2000 μg/ml without metabolic activation, in the second experiment. There are deficiencies in this study that have influenced the results: (1) the metabolic activation system did not demonstrate an adequate efficiency; (2) the mean values of spontaneous mutation frequencies observed in the untreated controls differ by a factor of 10 according to the fact that the assay was conducted in the presence or in the absence of an activation system. The study is unsuitable for evaluation.

Ref. : 7

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

Guideline	:	/
Species/strain	:	Chinese hamster ovary cells
Replicates	:	2 independent tests
Test substance	:	IMEXINE FAC dissolved in dimethylformamide
Batch No.	:	Op. 10
Purity	:	/
Concentr. scored	:	200, 1000, and 2000 μg/ml with and without metabolic activation
GLP	:	In compliance

HC Orange No. 3 has been investigated for induction of chromosomal aberrations in CHO cells. Liver S9 fraction from rats pretreated with β-naphthoflavone/phenobarbitone was used as the exogenous metabolic activation system. Test concentrations were selected such that the highest concentration resulted in a mitotic index in the range of 50% of control.

Results
There were no statistically significant increases over control in the aberration frequency of any cultures with or without S9. The study does not indicate the exposure times in the presence and in the absence of S9. Historical control values are not included. The study is inadequate.

Ref. : 8

2.8.2. Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline	:	/
Species/strain	:	Mouse, CD1 outbred
Group size	:	15 males + 15 females
Test substance	:	IMEXINE FAC suspended in 0.5% aqueous carboxymethylcellulose
Batch No.	:	Op. 10
Purity	:	/
Dose levels	:	0, 500, 1000, and 2000 mg/kg bw, i.p.
Sacrifice times	:	24, 48, and 72 hours
GLP	:	In compliance

HC Orange No. 3 has been investigated for induction of micronuclei in the bone marrow cells of mice. A preliminary study showed no signs of toxicity up to a dose of 2000 mg/kg bw/day, and hence this was used as the highest dose for the main study.

Results
There was no evidence of increased incidence of micronucleated polychromatic erythrocytes in any of the test groups. There was no change in the ratio of polychromatic to normochromatic erythrocytes. The positive control agent gave the expected result at 24 hours.
The study was conducted adequately but failed to demonstrate exposure to the bone marrow. Therefore, the test is unsuitable for the evaluation of the *in vivo* genotoxicity.

Ref. : 9

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

Guideline	:	OECD 486 (1997)
Species/strain	:	Wistar rats
Group size	:	4 males
Test substance	:	IMEXINE FAC in 0.5 % carboxymethylcellulose
Batch No.	:	T10
Purity	:	/
Dose levels	:	500 and 2000 mg/kg bw, by single gavage
Exposure time	:	16 hours, all dose groups; 2 hours, high dose group
GLP	:	In compliance

HC Orange No. 3 has been investigated for induction of unscheduled DNA synthesis in Wistar rats hepatocytes at 2 doses, 500 and 2000 mg/kg bw. Positive controls are in accordance with OECD guideline and UDS analysed by autoradiography. Four males were used per dose/time sampling.

Results
Animals expressed toxic reactions. No substantial difference in viability of treated rat hepatocytes was observed as compared to controls. No dose level of the test agent expressed evidence of UDS induction in the hepatocytes of the treated animals as compared to the concurrent vehicle control group. No substantial shift to a higher value was observed in the percentage distribution of the nuclear grain counts.
The positive control agents gave positive results at both 2 hours and 16 hours. However, a large interindividual variability regarding the expected values in the net nuclear grain counts was noted in the 2-hour treatment: one animal with a value lower than the lower limit; another with a value higher than the higher limit. However, this does not invalidate the assay, the net grain counts being positive in both cases.

This study is adequate and the results negative. HC Orange No. 3 did not induce DNA damage leading to UDS in hepatocytes of *in vivo* treated rats.

Ref. : 12

2.9 Carcinogenicity

No data

2.10 Special investigations

No data

2.11. Safety evaluation

NOT APPLICABLE

2.12. Conclusions

HC Orange No. 3 is a secondary alkanolamine, and thus, it is prone to nitrosation. No information on the nitrosamine content in the dye and dye formulations has been provided. No experimental data on stability are provided.
Purity of the chemical reported for one batch only (Batch Op. 10) : it would be advisable to have an statement of the range of impurities that be may be present, based on the analysis of more than one batch.

HC Orange No. 3 was not toxic following acute administration to rats at 1000 mg/kg bw.
From a 13-week oral study in rats, a NOAEL of 100 mg/kg bw/day was concluded, based upon observed changes in various organ weights. The study also indicated staining of the eye by the dye. This may be a reversible staining effect.
HC Orange No. 3 was not toxic to the pregnant rat and gave no evidence of embryotoxicity or teratogenicity at doses up to 1000 mg/kg bw/day.

HC Orange n° 11 was non-irritating to rabbit skin and to the rabbit eye. Sensitisation potential has not been adequately tested.

Under the experimental conditions described in the percutaneous absorption study, most of the hair dye applied on the skin surface was removed with the washing procedure. Almost no diffusion in the receptor fluid was observed.

HC Orange No. 3 induced gene mutations in bacteria. The studies on mammalian cells *in vitro* (gene mutation and chromosome aberrations) were inadequate and not suitable for evaluation. The mouse bone marrow micronucleus test was not suitable for evaluation. The UDS test *in vivo* was adequate for evaluation and gave negative results. In the absence of results from the two *in vitro* mammalian cell tests, no conclusions can be drawn on the mutagenic/genotoxic potential of this compound.

2.13. References

1. Toxicol Labs Ltd, UK. Report LRL/54/93 (April 1994)
2. Toxicol Labs Ltd. Report No. A/E/38532 (November 1993)
3. Toxicol Labs Ltd, UK. Report No. A/S/38531 (June 1993)
4. Institut Francais de Toxicologie, France. Report No. 406331 (June 1984)
5. Toxicol Labs Ltd, UK. Report No. LRL/34/94 (March 1995)
6. Toxicol Labs Ltd, UK. Report No. M/AMES/38533 (January 1994)
7. Toxicol Labs Ltd, UK. Study Ref: M/ PML/39195 (September 1994)
8. Toxicol Labs Ltd, UK. Study Ref: M/CCA/38535 (July 1994).
9. Toxicol Labs Ltd, UK. Report No. M/MMN/38534 (September 1994)
10. Toxicol Labs Ltd, UK. Report No. LRL/36/94 (October 1994)
11. L'Oreal, France. Ref: 92/12/1A, 92/12/1B, 92/12/08A, 92/12/08B (January 1993)

12. Cytotest Cell Research GmbH and Co. KG (CCR) D-Rossdorf. In vivo/in vitro Unscheduled DNA Synthesis in Rat Hepatocytes with Imexine FAC. Report n° 676900, 12 January 2001
13. L'Oréal Recherche, France. In vitro percutaneous absorption of Imexine FAC (COLIPA B68). Study report 16089, 03/05/2001

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, Log Pow, experimental data on stability, sensitivity to light and moisture.

* nitrosamine content in various batches of HC Orange No. 3 and in the hair dye formulations containing this chemical;

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

4. Other considerations

/

5. Minority opinions

/