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

CONCERNING

# HC RED Nº 8

COLIPA n° C119

adopted by the SCCNFP during the 23rd plenary meeting
of 18 March 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 Red n° 8 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 Red No. 8 (INCI name)

### 2.1.2. Chemical names

| | | |
|---|---|---|
| Chemical name | : | 1-[(3-aminopropyl)-amino]-anthraquinone, hydrochloride |
| CAS name | : | 9,10-anthracenedione, 1-[(3-aminopropyl)amino]-, hydrochloride |
| Synonyms | : | 1-aminopropylaminoanthraquinone, HCl<br>1-(3-aminopropylamino)anthraquinone, HCl<br>chlorhydrate de N-(amino-3 propyl)amino-1-anthraquinone<br>aminopropylamino-9,10-anthraquinone, chlorhydrate<br>1-aminopropylamino-9,10-anthraquinone, chlorhydrate<br>1-[(3-aminopropyl)amino]-9,10-anthracenedione, hydrochloride |

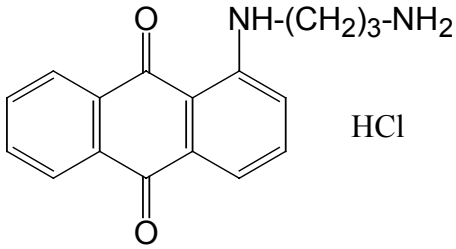
### 2.1.3. Trade names and abbreviations

| | | |
|---|---|---|
| Trade name | : | IMEXINE® BG (Chimex) |
| COLIPA No. | : | C-119 |

### 2.1.4. CAS n°/EINECS n°

| | | |
|---|---|---|
| CAS n° | : | 13556-29-1 (free base)<br>97404-14-3 (hydrochloride) |
| EINECS n° | : | 306-778-0 |

### 2.1.5. Structural formula




### 2.1.6. Empirical formula

| | | |
|---|---|---|
| Emp. formula | : | $C_{17}H_{16}N_2O_2$, HCl |
| Mol. weight | : | 316.79 |

## 2.1.7. Purity, composition, and substance codes

All analytical data relate to Batch op. 11.

Purity

| | | |
|---|---|---|
| Titre as determined by alkalinity | : | 89.6% |
| Chloride ions | : | 11.2% (theoretical value = 11.5%) |
| Water content | : | 0.4% |
| Ash content | : | < 0.2% |
| Heavy metals | : | < 20 ppm |

Impurities and reaction intermediates

| | | |
|---|---|---|
| 1-chloro-anthraquinone | : | 0.35% |
| anthraquinone | : | 0.11% |
| 1-amino-anthraquinone | : | 0.084% |
| 2-chloro-anthraquinone | : | 0.081% |
| 1,5-bis-(3-amino-propyl-amino)-anthraquinone | : | 4.4% |
| 1,8-bis-(3-amino-propyl-amino)-anthraquinone | : | 0.32% |
| [1-((3-aminopropyl)-amino)-anthraquinone]-bis | : | (not quantified; no available standard) |

Two unknown trace impurities detected by HPLC; not quantifiable

Solvent residues

| | | |
|---|---|---|
| isopropanol | : | 0.37% |
| methyl ethyl ketone | : | 0.085% |

## 2.1.8. Physical properties

| | | |
|---|---|---|
| Subst. Code | : | Colipa C-119 |
| Appearance | : | Red powder, almost odourless |
| Melting point | : | 280 °C |
| Boiling point | : | no information |
| Density | : | 0.4 g/cm$^3$ |
| Rel. vap. dens. | : | no information |
| Vapour Press. | : | no information |
| Log $P_{ow}$ | : | 1.8 (calculated) |
| pH | : | 6.4, at 0.1% in de-ionised water |
| Storage | : | At room temperature, protect from light |

HPLC, IR, UV-Vis, MS, and NMR features available for identification

## 2.1.9. Solubility

Soluble in water at 0.1% (with presence of slightly insoluble material)
Soluble in ethanol at 0.1% (with presence of slightly insoluble material)
Soluble in DMSO at 5%
Insoluble in 1,1,1-trichloroethane at 0.1%

**General comments on analytical and physico-chemical characterisation**

The following issues do not, or poorly comply with the basic requirements for proper characterisation :

* solubility information inadequate;
* the purity of the test material has been determined by alkalinity. Thus, the purity information is inadequate;
* HPLC characterisation of the test material is inadequate;
* anthraquinone and anthraquinone derivatives were detected as impurities; related health hazards unknown or not addressed in the submission;
* chemical purity not stated in a number of toxicity study reports;
* log $P_{OW}$ provided; experimental protocol not specified;
* no experimental data on stability available; suggestion of chemical sensitivity to light and moisture; at least on one occasion the stability in solvent reported as unknown.

## 2.2. Function and uses

COLIPA C-119 will be incorporated in semi-permanent hair dye formulations at a maximum concentration of 0.2%. It is common practice for 35 ml of undiluted formulation to be applied for a period of 30 minutes before washing. It is assumed that application may be repeated weekly.

# TOXICOLOGICAL CHARACTERISATION

## 2.3. Toxicity

### 2.3.1. Acute oral toxicity

| | | |
|---|---|---|
| Guideline | : | OECD 401 (1987) |
| Species/strain | : | Sprague Dawley rat ICO:OFA-SD (IOPS Caw) strain |
| Group size | : | 5 male + 5 female |
| Test substance | : | IMEXINE BG suspended in water |
| Batch no | : | op.11 (96% pure) |
| Dose | : | 2000 mg/kg bw. |
| Observ. period | : | 14 days |
| GLP | : | in compliance |

Groups of 5 male and 5 female rats received a single limit dose of test substance by gastric gavage. The animals were observed for mortalities and clinical signs for 14 days. Bodyweights were recorded at intervals and macroscopic abnormalities were recorded at autopsy.

Results
One animal of each sex exhibited clinical signs of toxicity (distended abdomen, piloerection, stiff gait and decrease in spontaneous activity) from day 4. Of these two animals, the male died on

day 6, and the female recovered, appearing normal by day 14. The remaining 8 animals exhibited no clinical signs except for hypersalivation 30 min after dosing, and their bodyweight gain was considered to be normal. At autopsy, no macroscopic abnormalities were reported for any of the animals (including the male that died). The cage bedding was reported to be red-coloured from day 2 to 5.
The oral LD50 was reported to be greater than 2000 mg/kg bw.

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 | : | Crl: CD (SD) BR strain rat |
| Group size | : | 10 male + 10 female |
| Test substance | : | IMEXINE BG in aqueous solution |
| Batch no | : | op.11 (96% pure) |
| Dose levels | : | 0, 5, 20 and 80 mg/kg bw/day, 7 days/week by gavage |
| Exposure period | : | 91 or 93 days |
| GLP | : | in compliance |

Groups of 10 male and 10 female rats were dosed with the test substance by gavage at 0, 5, 20 and 80 mg/kg bw/day, 7 days/week for 13 weeks. The dosing solutions were analysed before the start of the study for stability and monthly for verification of concentration. During the study, the animals were observed daily for clinical signs and mortality, and weekly for bodyweight and food consumption. In week 13 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 periods, a full autopsy was conducted with recording of weights and macroscopic and microscopic examination of major organs. Ophthalmological examination was conducted before the start and at the end of the study on controls and high dose animals.

Results
One male animal, dosed at 80 mg/kg bw/day, died on day 10 and this was considered to be probably due to a dosing error. Clinical signs of treatment were dose-related incidence and onset of hypersalivation at 20 and 80 mg/kg bw/day and loud breathing in some animals at 80 mg/kg bw/day. Coloration of the urine, fur and/or extremities was noted at 20 and 80 mg/kg bw/day and was attributed to the staining properties of the substance. There were no significant differences between control and treated groups with respect to bodyweight gain, food consumption or efficiency of food utilisation. Ophthalmological examinations revealed no abnormalities in any of the animals.

Slight differences in haematological parameters (mean cell haemoglobin, prothrombin time and fibrinogen level) and biochemical parameters (chloride, calcium and urea) were not considered to be of toxicological importance because individual values were within the normal range. The plasma of some females given 80 mg/kg bw/day was pink-coloured. Urinary parameters showed no difference between controls and test groups. Urine of all treated animals was pink, which was attributed to the colour of the dye.
Absolute and relative liver weights were significantly increased in male rats at 20 and 80 mg/kg bw/day (absolute weights: 116 and 125% of control; relative weights: 113 and 130% of control, respectively). A slight increase in relative liver weight was also seen in the high dose females (108% of control). In addition, the absolute and relative kidney weights were increased in male rats at 20 and 80 mg/kg bw/day (absolute weights: 112 and 117% of control; relative weights: 109 and 122% of control, respectively). The difference in absolute kidney weight was not statistically significant for the 20 mg/kg bw/day group.

At autopsy, liver enlargement was noted in 1/10 males at 20 mg/kg bw/day and 6/10 males at 80 mg/kg bw/day. Kidneys had a grey/green or irregular colour in 3/10 males at 20 mg/kg bw/day and 4/10 males at 80 mg/kg bw/day. Discoloration was noted in the gastrointestinal and urinary tracts of some animals at these doses and was attributed to the staining properties of the hair dye.

Histological examination revealed minimal to moderate hepatocellular hypertrophy in 6/10 males treated at 80 mg/kg bw/day. Because there were no associated degenerative or necrotic changes the authors concluded that this was an adaptive response. Furthermore, based on the absence of histo-pathological changes, they concluded that the observed liver enlargement was not of toxicological importance in female rats, or in male rats dosed at 20 mg/kg bw/day.
Accumulation of acidophilic globules was seen in the cortical tubular epithelium of kidneys in 1/10 control males (minimal), 4/10 males at 5 mg/kg bw/day (minimal), 8/10 males at 20 mg/kg bw/day (minimal to moderate) and 9/10 males at 80 mg/kg bw/day (minimal to moderate). It was not associated with degenerative or necrotic changes. These changes were considered to be due to accumulation of $\alpha$2micro globulin, which is a male rat-specific phenomenon. The authors concluded that the NOAEL was 20 mg/kg bw/day.
The dose-related increase in liver weight cannot be clearly defined as a toxicological response. Nevertheless, a cautious approach would lead to the conclusion that 20 mg/kg/day is a LOAEL and the lower dose of 5 mg/kg/day should be viewed as the NOAEL.

Ref. : 5

## 2.3.8. Sub-chronic dermal toxicity

No data

### 2.3.9. Sub-chronic inhalation toxicity

No data

### 2.3.10. Chronic toxicity

No data

## 2.4. Irritation & corrosivity

### 2.4.1. Irritation (skin)

| | | |
|---|---|---|
| Guideline | : | / |
| Species/strain | : | New Zealand albino rabbit |
| Group size | : | 6 males |
| Test substance | : | IMEXINE BG suspended at 1% in propylene glycol |
| Batch no | : | op.4 (purity not stated in study report) |
| Dose | : | 0.5 ml |
| GLP | : | not in compliance |

The substance was dissolved in water at a concentration of 1%, and 0.5 ml was applied to two 2cm square areas of intact skin of 6 male rabbits. Occlusive patches were applied and left in place for 23 hours. Biopsies were taken at 1 and 48 hours after removal of the patches for histological examination.

Results
The coloration of the skin prevented evaluation of erythema, and therefore histological examinations were required. No pathological reactions were reported and the substance was classified as non-irritating to rabbit skin at a concentration of 1% in propylene glycol.

Ref. : 3

### 2.4.2. Irritation (mucous membranes)

| | | |
|---|---|---|
| Guideline | : | Journal Officiel de la République Française of 21/4/71 and 5/6/73 |
| Species/strain | : | New Zealand albino rabbit |
| Group size | : | 6 (sex not specified) |
| Test substance | : | IMEXINE BG suspended at 1% in water |
| Batch no | : | op.4 (purity not stated in study report) |
| Dose | : | 0.1 ml |
| GLP | : | not in compliance |

The test substance was suspended in water at a concentration of 1% and 0.1 ml was applied once to the left eye of 6 rabbits, without rinsing. The right eye served as control and was untreated. Ocular reactions were recorded at 1 hour and 1, 2, 3, 4, and 7 days after instillation.

Results
The red colour of the substance interfered with evaluation of erythema at one hour. Mild reactions of the conjunctiva and iris were observed in all rabbits within 24 hours of instillation.

There were no corneal reactions. The Acute Ocular Irritation Index was calculated as 11 out of 80 and the substance was classified as slightly irritating to the rabbit eye at a concentration of 1% in water.

Ref. : 2

## 2.5. Sensitisation

**Epicutaneous maximisation test**

| | | | |
|---|---|---|---|
| Guideline | : | / | |
| Species/strain | : | Albino Hartley guinea pig | |
| Group size | : | 10 male + 10 female (no control group) | |
| Test substance | : | IMEXINE BG tested neat | |
| Batch no | : | op.5 (purity not stated in study report) | |
| Concentrations | : | intradermal induction : | 0.1 ml 50% Freund's complete adjuvant (FCA), on days 1 and 10 |
| | | topical induction : | 0.3 g neat test substance for 48 hours occluded, on 10 occasions |
| | | challenge : | 0.3 ml neat test substance for 48 hours, occluded |
| GLP | : | not in compliance | |

The test was performed according to Brulos et al, cited in reference n° 4.
Induction consisted of intradermal injections of FCA on days 1 and 10 and topical applications of 0.3g of the neat test substance under occlusive patch to the shoulder for 48 hours on days 1, 3, 5, 8, 10, 12, 15, 17, 19 and 22. An interval of 12 days was allowed after induction and then the animals were challenged by a single 0.3g topical application of the neat test substance under occlusive patch on the flank for 48 hours. The skin was examined 1, 6, 24 and 48 hours after removal of the challenge patches for erythema and oedema.

Results
Two guinea pigs died (one of each sex). The cause of death was not established, but was not considered to be treatment-related.
Cutaneous examination revealed oedema in 3 animals at 1 hour, and in one (different) animal at 6 hours. Red staining of the skin due to the hair dye prevented macroscopic assessment of erythema. The test did not conform with OECD guideline 406.

Ref. : 4

## 2.6. Teratogenicity

| | | |
|---|---|---|
| Guideline | : | OECD 414 (1981) |
| Species/strain | : | Sprague-Dawley rat, Crl: CD (SD) BR strain |
| Group size | : | 25 females (mated) |
| Test substance | : | IMEXINE BG in aqueous solution |
| Batch no | : | op.11 (purity 101.6%) |
| Dose levels | : | 0, 10, 30 and 100 mg/kg bw/day |
| Treatment period | : | Days 6 to 15 of pregnancy, inclusive |
| GLP | : | in compliance |

Groups of 25 female rats were dosed with the test substance by gavage at 0, 10, 30 and 100 mg/kg bw/day on days 6 to 15 after mating. The dams were observed daily for clinical signs and

mortality, bodyweight and food consumption were recorded on days 0, 2, 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 bodyweight, sex and macroscopic external observations, and for skeletal and visceral abnormalities (half for each endpoint). The concentrations of the dosing formulations were verified analytically on the first and last day of dosing.

Results
There were no premature deaths or abortions and no treatment-related clinical signs except for redness of the urine and faeces in all high dose animals from day 7 to 16. The 30 and 100 mg/kg bw/day dose groups significant reduction in weight gain over the period day 6-9 (19 and 14 g, respectively, vs 23 g for controls). Food consumption was also decreased over this period in the high dose group only, but actual body weight did not differ significantly at any time point.
No macroscopic observations were reported for the treated dams at autopsy. The mean numbers of corpora lutea, live foetuses, sex distribution and the mean foetal bodyweights were comparable for control and treated groups. The post-implantation loss was similar to controls for the 10 and 30 mg/kg bw/day groups but was slightly higher for the high dose group (5.3% vs 2.0% in controls).
There were no dose-related increases in foetal abnormalities or malformations. Some individual significant differences were observed with very low incidence, which were within the historical control range and not considered to be treatment- related.
There was evidence of maternal toxicity at 30 and 100 mg/kg/day, and of embryo-toxicity at 100 mg/kg/day, but no teratogenicity. The NOAEL was 10 mg/kg/day for the pregnant female rat and 30 mg/kg/day for embryo-foetal development.

Ref. : 11

## 2.7. Toxicokinetics (incl. Percutaneous Absorption)

### 2.7.1 Percutaneous Absorption *in vitro*

**Study 1**

| | | |
|---|---|---|
| Guideline | : | / |
| Tissue | : | Human mammary epidermis, heat-separated |
| Method | : | Franz diffusion cell (static) |
| Test substance | : | IMEXINE BG, 0.15% in formulation |
| Batch no | : | op.11 (purity not stated in study report) |
| Dose levels | : | c. 40mg formulation in the presence/absence of 10 mg hair |
| Replicate cells | : | 7 cells without hair and 8 cells with hair |
| GLP | : | not in compliance |

The skin penetration of COLIPA C119 was evaluated in a static Franz diffusion cell system. Human epidermis was prepared by heat-separation from previously frozen mammary skin. The test substance was prepared at a concentration of 0.15% in a formulation. Approximately 40 mg of the mixture was applied to 2cm$^2$ of epidermal membrane with and without addition of 10 mg finely chopped bleached hair for 30 minutes and then excess washed off with 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 by means of addition of Chinese ink at the end of the study. Any cells showing penetration of the ink were eliminated from the analysis.
*Results:*
The quantity of test substance penetrating through the epidermis to the receptor fluid was close to, or below, the limits of detection. Maximum penetration corresponded to 0.016% of applied dose in the presence of hair and 0.017% in the absence of hair.
This study used a concentration of hair dye that is lower than the intended use level. Recovery of the test substance was not determined. Physiological saline was used as the receptor fluid which may not be appropriate for a substance with poor water solubility. A longer period should be allowed for permeation of substance from the epidermis into the receptor fluid.
The study is considered inadequate (see SCCNFP Notes of Guidance).

Ref. : 12

**Study 2**

| | | |
|---|---|---|
| Guideline | : | / |
| Tissue | : | Human abdominal (kept frozen at - 18°C) dermatomed skin |
| Skin integrity | : | TEWL measurement |
| Method | : | Franz diffusion cell (static) |
| Test substance | : | 1-[(3-aminopropyl)-amino]-anthraquinone, hydrochloride (IMEXINE BG) at the concentration of 0.15 % w/w in commercial type formulation batch number 443824 (HPLC control of the concentration) |
| Batch no | : | 000012, (purity not stated in study report) |
| Dose of formulation applied | : | 20 mg/cm² |
| Replicate cells | : | 4 skin donors, 2 cells/donor, 8 cells interpreted (9 cells performed, one discarded because total recovery outside the limits of acceptance |
| Analytical method | : | HPLC (validated) with UV-Visible detection |
| Limit of detection | : | not indicated |
| limit of quantitation | : | 0.1 µg/ml for receptor fluid, 5 ng/ml for the other samples |
| Solubility in the receptor | : | verified by spectrophotometry 0.008 mg/ml |
| Stability of the ingredient | : | no information concerning the stability of IMEXINE BG in the formulation according to time |
| GLP | : | in compliance |

The skin penetration of COLIPA C119 was evaluated in a static Franz diffusion cell system. Human abdominal skin previously frozen was dermatomed to a constant thickness (375 ± 40 µm). The integrity of the skin was evaluated by the measurement of the TEWL, the skin surface temperature was monitored (31.6 ± 0.5 °C). The solubility of COLIPA C119 in the receptor fluid (physiological saline) was checked in the range of the concentration used. The test substance was prepared at a concentration of 0.15 % in a "commercial type" formulation. A total of 20.46 ± 0.34 mg/cm² of the formulation i.e. 30.09 ± 0.40 µg/cm² (exactly measured by weight) were applied to 2 cm² 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 (9 to 20 strips), in the epidermis/ dermis and in the remaining skin outside the application area. After assay of

COLIPA C119 in the washing material (skin excess) the mass balance of the study was calculated (90.71 ± 3.62 % of the applied dose)

Results
The quantity of test substance penetrating through the skin to the receptor fluid was below the limit of quantification of the analytical method (< 0.02 µg/cm²).The amount recovered in the horny layer was also below the limit of quantification of the analytical method (< 0.03 µg/cm²).The epidermis and the dermis content was 0.40 ± 0.37 % of the applied dose (0.12 ± 0.11 µg/cm²). The recovery (mass balance) of the test substance was 90.71 ± 3.62 % of the applied dose.
The absorbed amounts of COLIPA C119 (epidermis + dermis + receptor fluid) represents 0.40 ± 0.37 % of the applied dose (0.12 ± 0.11 µg/cm²) 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 |
| Species/strain | : | *Salmonella typhimurium*, TA98, TA100, TA1535, TA1537 |
| Batch no | : | C3024 6B / OP 11 K1 (M.W. 316.5) |
| Purity | : | 101.6 % by NaOH titer |
| GLP | : | not in compliance |

The maximum dose tested was 150 µg/plate because of toxicity

Liver S9 fraction from Wistar liver rats pre-treated with Aroclor 1254 was used as the exogenous metabolic activation system.

Results

| | | | | |
|---|---|---|---|---|
| In the first experiment | : | - S9 | : | Positive in TA1537 |
| | | + S9 | : | Positive in one dose in TA1537 |
| Second experiment | : | - S9 | : | Positive in TA1537 |
| | | + S9 | : | Positive in TA1537 |

Conclusions
Based on the reversion rate, it is concluded that the test agent C 119 shows evidence of mutagenic activity in this bacterial test system in the presence or in the absence of metabolic activation system in the tester strain TA 1537 (frameshift).

Ref. : 6

***In Vitro* Mammalian Cell Gene Mutation Test**

| | | |
|---|---|---|
| Guideline | : | / |
| Species/strain | : | mouse lymphoma L5178Y cells/ HPRT Locus |
| Replicates | : | 2 independent tests with and without metabolic activation |

| | | |
|---|---|---|
| Test substance | : | Imexine BG |
| Batch no | : | OP 11 |
| Treatment time | : | 2 hours |
| Purity | : | not given |
| GLP | : | in compliance |

Liver S9 fraction from liver rats pre-treated with Aroclor 1254 was used as the exogenous metabolic activation system.

Results
The compound shows some slight positive effects observed at low doses (up to 12 µg/ml – upper doses being toxic)) in the presence of S9 in both experiments. According to the authors, no biologically significant relevance was associated with the positive results obtained.

Conclusions
Based on the mutation frequency rate observed, the results are equivocal.

Ref. : 15

**_In Vitro_ Mammalian Chromosome Aberration Test**

| | | |
|---|---|---|
| Guideline | : | / |
| Species/strain | : | Chinese Hamster Ovary (CHO) cells |
| Replicates | : | No |
| Test substance | : | batch no C3024 GB |
| Exposure time | : | 1 h |
| GLP | : | not in compliance |

Results
COLIPA C119 has been investigated for induction of chromosomal aberrations in Chinese Hamster Ovary cells. Liver S9 fraction from rats (origin not described) was used as the exogenous metabolic activation system.
The preliminary toxicity study was not performed at the same time; no raw data are given and no replicate experiment was done. The exposure time of cells to the test agent is too short and does not allow an accurate evaluation.

Conclusions
This study is not adequate.

Ref. : 8

**DNA Damage and Repair-Unscheduled DNA Synthesis-Mammalian Cells _In Vitro_**

| | | |
|---|---|---|
| Guideline | : | OECD 482 |
| Species/strain | : | HeLa cells |
| Replicates | : | Yes |
| Test substance | : | Purity 101.6 % given by NaOH titer |
| Exposure time | : | 2.5 h |
| GLP | : | in compliance |

Results
COLIPA C119 has been investigated for induction of unscheduled DNA synthesis in HeLa cells. Liver S9 fraction from liver rats pre-treated with Aroclor 1254 was used as the exogenous metabolic activation system.
No increase (expressed as dpm/µg DNA) in UDS was noted in both experiments. However, the methodology adopted (scintillation counting) is the less sensitive to be used in this type of test, according to the scientific literature.

Conclusions
This study is not adequate.

Ref. : 9

### 2.8.2. Mutagenicity/Genotoxicity *in vivo*

***In Vivo* Mammalian Erythrocyte Micronucleus Test**

| | | |
|---|---|---|
| Guideline | : | OECD 474 |
| Species/strain | : | Mouse, Crl:CD-1 mice |
| Group size | : | 5 male + 5 female |
| Test substance | : | Imexine BG, batch OP 11 |
| Batch no | : | purity: not given |
| Dose levels | : | 32.25 , 62.5 and 125 mg/kg bw, single intraperitoneal injection |
| Sacrifice times | : | 24, 48 and 48 hours after dosing |
| GLP | : | in compliance |

COLIPA C119 has been investigated for induction of micronuclei in the bone marrow cells of CD-1 mice. The substance was administered once by single intraperitoneal injection at 0, 32.25, 62.50 and 125 mg/kg bw and the bone marrow harvested after 24 ,48 and 72 hours. Negative and positive controls were in accordance with the OECD guideline.

Results

Mean values of micronucleated PCE
No statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic cells over the concurrent vehicle control values were observed .

PCE/NCE ratio
Groups of mice treated with COLIPA C119 did exhibit a slight variation of the PCE/NCE ratio dose dependent at 24 H sacrifice time. No other variation was found.

Conclusions
Under the conditions of the test, it can be concluded that there was no evidence of induced chromosomal or other damage leading to the micronucleus formation in polychromatic erythrocytes treated mice .
However, there is no clear evidence that the test agent has reached the target organ.

Ref. 13

**General Conclusions**

* C119 has been tested in bacterial cells for gene mutation in 4 tester strains. The results of the bacterial gene study clearly demonstrated the mutagenic properties in strain TA 1537 at the gene level.
* The *in vitro* test for mammalian gene mutation assay has yielded equivocal results.
* The *in vitro* test for clastogenicity in CHO cells is considered inadequate.
* The UDS on HeLa cells is considered inadequate.
* The *in vivo* micronucleus test in mice gave negative results; no firm evidence that the bone marrow was reached by the test agent was noted.

No conclusions can be drawn for mutagenicity.

## 2.9. Carcinogenicity

No data

## 2.10. Special investigations

No data

## 2.11. Safety evaluation

**NOT APPLICABLE**

CALCULATION OF THE MARGIN OF SAFETY

HC Red n° 8

(Semi-permanent)

Based on a usage volume of 35 ml, containing at maximum … %

| | | | |
|---|---|---|---|
| Maximum absorption through the skin | A (µg/cm$^2$) | = | µg/cm$^2$ |
| Typical body weight of human | | = | 60 kg |
| Skin Area surface | SAS (cm$^2$) | = | cm$^2$ |
| Dermal absorption per treatment | SAS x A x 0.001 | = | mg |
| Systemic exposure dose (SED) | SAS x A x 0.001/60 | = | mg/kg |
| No observed adverse effect level (mg/kg) (species, study) | NOAEL | = | mg/kg |

| | |
|---|---|
| Margin of Safety | NOAEL / SED = |

## 2.12. Conclusions

COLIPA C119 was non-irritating to rabbit skin and slightly irritating to the rabbit eye when applied at a concentration of 1%. It has not been adequately tested for sensitisation.

A 13-week oral study in rats established that the substance produced evidence of hepato-toxicity at 80 mg/kg bw/day. There was also a dose-related increase in liver weight at 20 and 80 mg/kg bw/day. In the absence of histo-pathological abnormalities, increased liver weight cannot clearly be defined as adverse, but nevertheless a cautious approach indicates that the NOAEL should be viewed as 5 mg/kg bw/day. When administered during organogenesis, the substance slightly affected maternal food consumption and weight gain, with a NOAEL of 10 mg/kg bw/day. Embryotoxicity was seen at higher doses than maternal toxicity and therefore could be a secondary effect. There was no evidence of teratogenicity.

Percutaneous penetration has been investigated following the SCCNFP Notes of Guidance using human skin *in vitro*. The maximum penetration rate after a contact with the skin of 30 minutes and a diffusion time of 24 hours, established by this study is 0.4 % of the applied dose (i.e. 0.12 µg/cm²).

When C119 was tested in bacterial cells for gene mutation in 4 tester strains, the results of the bacterial gene study clearly demonstrated the mutagenic properties in strain TA 1537 at the gene level. The results at the *in vitro* test for mammalian gene mutation assay is negative, but the results are equivocal. The *in vitro* test for clastogenicity in CHO cells is considered inadequate. The UDS on HeLa cells is considered inadequate. The *in vivo* micronucleus test in mice gave negative results; no firm evidence that the bone marrow was reached by the test agent was noted.

No conclusions can be drawn for mutagenicity.

## 2.13. References

1. CIT, France. Study No: 9202 TAR (Oct 1992)
2. IFREB, France. Report No: 801238 (Jan 1978)
3. IFREB, France. Report No: 760602 (June 1976)
4. IFREB, France. Report No: 811311 (Nov 1978)
5. CIT, France. Study No: 9762 TCR (Nov 1993)
6. Microtest Res. Ltd, UK. Study No: BRP 8/S (June 1988)
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8. University of Leiden, NL. Report of 8th Dec 1988
9. Microtest Research Ltd, UK. Report BRP 8/He (June 1988)
10. Toxicol Labs, UK. Study No: M/MMN/35147 (Feb 1993)
11. CIT, France. Study No. 10360 RSR (Dec 1993).
12. L'Oreal, France. Ref: 90/08/643, 90/08/644 (Jan 1991)
13. RCC-CCR project n° 689100, July 04 2001, In vivo/in vitro unscheduled DNA synthesis in rat hepatocytes with IMEXINE BG
14. ADME Bioanalysis, In vitro percutaneous absorption of IMEDINE BG, report study n° ERO/IMEX/0001, 27/10/00

## 3. Opinion of the SCCNFP

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

Consequently, and before any further consideration, the following are required :

data on the genotoxicity/mutagenicity following the SCCNFP-opinion “Proposal for a Strategy for Testing Hair Dye Cosmetic Ingredients for their Potential of Genotoxicity / Mutagenicity”, doc. n° SCCNFP/0566/02 of 4 June 2002, and in accordance with the Notes of Guidance, regularly updated by the SCCNFP (doc. n° SCCNFP/0321/00).

## 4. Other considerations

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## 5. Minority opinions

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