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

CONCERNING

4-AMINO-2-NITRODIPHENYLAMINE-2'-CARBOXYLIC ACID

COLIPA n° B87

adopted by the SCCNFP during the 23rd plenary meeting
of 18 March 2003

1. Terms of Reference

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 2-Nitro-4-amino-diphenylamine-2'-carboxylic acid 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

2-Nitro-4-amino-diphenylamine-2'-carboxylic acid (INCI name)

2.1.2. Chemical names

Chemical name : 2-[(4-Amino-2-nitrophenyl)-amino]-benzoic acid
CAS name : 2-Nitro-4-amino-diphenylamine-2'-carboxylic acid
Synonyms : /

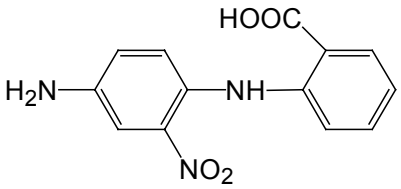
2.1.3. Trade names and abbreviations

Trade name : RO1082
COLIPA No. : B-87
Colour Index : Not Given

2.1.4. CAS n° / EINECS n°

CAS n° : 117907-43-4
EINECS n° : /

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{13}H_{11}N_30_4$
Mol. weight : 273.3

2.1.7. Purity, composition, and substance codes

Purity
Determined by HPLC (Batch 3279/114) : 98%

Impurities and reaction intermediates (Batch 3279/114)

Anthranilic acid	:	up to 2%
Unidentified fluorine compounds	:	0.07%

2.1.8. Physical properties

Subst. Code	:	COLIPA B-87
Appearance	:	Dark red crystals, odourless
Melting point	:	> 218 °C, with decomposition
Boiling point	:	No information
Density	:	No information
Rel. vap. dens.	:	No information
Vapour Press.	:	No information
Log P_{ow}	:	No information
Storage	:	Protect from light and moisture

2.1.9. Solubility

Slightly soluble in ethanol.

General comments on analytical and physico-chemical characterisation

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

* purity of the chemical assessed reliably (HPLC) and reported for only one batch: it would be advisable to have an official measure of purity consistency based on the analysis of more than one batch;
* low-level fluorine compounds detected but not identified;
* no mention of solvent residues, if any;
* with reference to Submission 1, SCC NFP asked for appropriate information on purity : no additional data available from Submission 2;
* chemical purity not stated in a number of toxicity study reports;
* some relevant physico-chemical parameters (in particular, log P_{OW}) unassessed/unreported;
* no experimental data on stability provided,
* information on solubility inadequate.

2.2. Function and uses

COLIPA B87 will be incorporated into semi-permanent hair dyes at a maximum concentration of 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 at weekly intervals.

In addition, the substance will be incorporated into oxidative hair dyes at a maximum concentration of 4%. Dilution with hydrogen peroxide will result in a maximum applied

concentration of 2%. It is common practice for 100 ml of formulation to be applied for a period of 30 minutes before washing. It is assumed that application may be repeated at monthly intervals.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Guideline	:	/
Species/strain	:	Wistar Albino rat
Group size	:	5 male + 4 female
Test substance	:	Ro 1082 suspended in aqueous solution of 1% carboxymethylcellulose and 0.5% Cremophor
Batch no	:	2495/127 (purity not stated in study report)
Dose	:	2000 mg/kg bw
Observ. Period	:	14 days
GLP	:	in compliance

Groups of 5 male and 4 female received a single dose of test substance at 2000 mg/kg bw by gastric gavage. The animals were observed daily and body weights were recorded on days –1, 0, 2, 7 and 14 of the study. Macroscopic examination of main organs was performed at autopsy. No histological examinations were performed.

Results
There was one death in the male group which was assumed to be treatment-related (time after dosing not specified). Autopsy observations were discoloration of the intestines, subcutis and muscles and lung oedema. Body weight gain for surviving animals was considered normal for the age and strain of rat. No abnormal findings were reported at scheduled autopsy. The LD50 was reported to be greater than 2000 mg/kg bw.

Ref. : 1

2.3.2. Acute dermal toxicity

Guideline	:	OECD 402 (1987)
Species/strain	:	Wistar Albino rat; Outbred,SPF
Group size	:	5 male + 5 female
Test substance	:	Ro 1082 suspended in 1% aqueous carboxymethylcellulose
Batch no	:	3962/46 (purity >95%)
Dose	:	2000 mg/kg bw, occluded patch, 24 hours
Observ. period	:	14 days
GLP	:	in compliance

Groups of 5 male and 5 female received a single dose of test substance at 2000 mg/kg bw. occlusively applied to an area of 25cm^2 for males and 18 cm^2 for females. The patches were left in place for 24 hours and the residue was removed with moistened tissue. The animals were

observed 1, 2 and 4 hours after dosing and thereafter daily for 14 days. 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 mortalities. Lethargy was noted in the majority of animals during the first 48 hours. Body weight gain was considered to be low for the majority of animals during the first week of the study period and in one female in the second week. No skin irritation was observed on the exposed skin but discoloration due to the compound was noted throughout the study period. No abnormalities were noted at autopsy. The dermal LD50 was reported to be in excess of 2000mg/kg bw in both males and females.

Ref. : 2

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 rat, CD SPF strain
Group size	:	10 males + 10 females plus 5 males and 5 females reversibility group
Test substance	:	Ro 1082 suspended in aqueous solution of 1% CMC and 0.5% Cremophor
Batch no	:	2495/161 (purity not stated in study report)
Dose	:	0, 20, 60 and 180 mg/kg bw/day
Exposure period	:	13 weeks (5 days per week)
GLP	:	in compliance

Groups of 10 male and 10 female rats were dosed with the test substance by gavage at 20, 60 and 180 mg/kg bw/day, 5 days a week for 13 weeks. The dosing solutions were analysed during weeks 1, 12 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 and water consumption. During weeks 6 and 13, blood was sampled for haematology and blood biochemistry. At the end of the treatment period a full autopsy was conducted with recording of weights of the adrenals, thymus, spleen, heart, kidney, brain, gonads and liver, 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
There were no mortalities and no clinical signs of toxicity. Staining of the fur, tail and urine was reported for all treatment groups. The body weight gain and food consumption were comparable for all dose groups. The water consumption was increased in female animals in a dose-related manner at 60 and 180 mg/kg bw/day.
There was a slight dose-related increase in the number of thrombocytes in male and female animals at week 13, which was significantly different from control at 180 mg/kg bw/day. Other minor significant differences in haematological parameters, as well as those seen in biochemical parameters were not dose-related and were within the normal range and therefore not considered to be of toxicological significance. No abnormal findings were reported in the ophthalmological examinations.
The absolute but not relative liver weights were increased in all female test groups without any relationship to dose. No other effects on organ weights were noted. Yellowish pigment was noted in the liver cells of some animals of all groups including the control and recovery groups. Other minor histo-pathological changes also showed a similar distribution between control and treated groups. The authors concluded that the NOAEL was reported to be 180mg/kg bw/day. The significance of the pigment in hepatocytes is not clear since it occurred in all dose groups, including controls. It is not clear why the authors disregarded the dose-related, significant increase in thrombocytes, and therefore the dose of 60 mg/kg bw/day should be viewed as the NOAEL.

Ref. : 8

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)

Single dose rabbit study

Guideline	:	OECD 404 (1981)
Species/strain	:	Albino rabbits, Kleinrusse strain (Chbb:HM)
Group size	:	5 males
Test substance	:	Ro 1082
Batch no	:	2495/127 (purity not stated in study report)
Dose	:	0.6g

GLP : in compliance

The substance (0.6 g moistened with water) was applied to a 6.25cm^2 area of intact skin of 5 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 and the effects were scored according to the Draize criteria.

Results
No skin reactions were observed. The neat substance was not irritating to rabbit skin.

Ref. : 4

Repeated application hairless mouse study

Guideline	:	/
Species/strain	:	Hairless mouse, hr/hr strain
Group size	:	5 females
Test substance	:	Ro 1082 in aqueous solution, adjusted to pH 8 with NaOH
Batch no	:	2495/127 (purity not stated in study report)
Dose	:	1-2 drops at 2% (week 1), 4% (week 2) and 8% (week 3)
GLP	:	in compliance

One to two drops of the substance were applied to the same area of skin of each animal once per day, 5 days per week, with increasing concentrations in 3 subsequent weeks. Animals were examined daily for signs of erythema and oedema and the observed effects scored according to Draize.

Results
No skin reactions were noted on the skin during or after the application period.

Ref. : 5

2.4.3. Irritation (mucous membranes)

Guideline	:	OECD 405 (1987)
Species/strain	:	Albino rabbits, Kleinrusse strain (Chbb:HM)/Fa
Group size	:	4 male
Test substance	:	Ro 1082
Batch no	:	2495/127 (purity not stated in the study report)
Dose	:	0.1g neat substance
GLP	:	in compliance

0.1g 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 1 and 6 hours and 1, 2, 3, 7, 10, 14, 17 and 21 days after instillation. The cornea was investigated further using fluorescein at 24 hours and 7 and 21 days.

Results
Instillation affected the cornea and conjunctivae. Slightly increased opacity of the cornea was seen in 2/4 rabbits eyes, resolving in one animal by day 4, but persisting to the end of the study

in the other animal. This observation was supported by the fluorescein examination which revealed slight corneal epithelial damage in these two animals. Mild to moderate irritation of the conjunctivae was seen in 4/4 animals and persisted to the end of the study period in one.
According to the defined criteria the pure test substance was classified as severely irritant to the rabbit eye.
The substance should be tested at concentration nearer to the in-use level to establish whether persistent damage occurs.

Ref. : 3

2.5. Sensitisation

Magnusson and Kligman study

Guideline	:	OECD 406 (1981)	
Species/strain	:	Pirbright White guinea pigs	
Group size	:	20 test + 20 control, females	
Test substance	:	Ro 1082 in aqueous solution or in Vaseline	
Batch no	:	2495/127 (purity not stated in study report)	
Concentration	:	intradermal induction :	0.1ml 50% Freund's complete adjuvant (FCA)
			0.1ml 0.5% aqueous test substance
			0.1ml 0.5% aqueous test substance/FCA
		topical induction :	0.5ml 20% test substance in Vaseline
		challenge :	0.2ml 10% aqueous test substance for 24 hours, occluded
GLP	:	in compliance	

A preliminary intradermal study indicated that 0.5% 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 test substance (20%) 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 (10%) 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 right 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 and 48 hours after removal of the challenge patches.

Results
After the first induction, all animals showed typical reactions to FCA. The substance provoked irritation in both induction periods. One animal died during the test period, possibly due to pneumonia. Twelve of the remaining 19 test animals showed slight to moderate erythema and oedema after the challenge. No effects were noted in control animals.
The test substance was a sensitiser to guinea pig skin.

Ref. : 6

Buehler study

Guideline	:	OECD 406 (1981)	
Species/strain	:	Pirbright White albino guinea pig	
Group size	:	20 female, tests + controls	
Test substance	:	Ro 1082 in vaselinum album	
Batch no	:	2495/161 (purity not stated in study report)	
Concentrations	:	topical induction :	3 x 0.2ml 20% test substance, 6 hours occluded
		Challenge :	0.2 ml 2.5% and 5% substance, 6 hours occluded
GLP	:	not in compliance	

Topical induction was by three 0.2 ml applications of test substance (20% in vaselinum album), for 6 hours under occluded patch to the right flank on three occasions (days 1, 8 and 15). The controls were untreated. An interval of 2 weeks was allowed after induction and then the animals were challenged by a single 0.2 ml topical application of the test substance (2.5% and 5% in vaselinum album) under occlusive patch on right and left flanks respectively for 6 hours. The skin was examined 24 and 48 hours after removal of the challenge patches.

Results
There was no evidence of erythema or oedema in any of the test animals after the challenge and the report concluded that the substance was not a sensitiser under the conditions of the test.

Ref. : 7

2.6. Teratogenicity

Guideline	:	OECD 414 (1981)
Species/strain	:	Wistar/HAN rat (Kfm:WIST,outbred, SPF)
Group size	:	25 females (mated)
Test substance	:	Ro 1082 suspended in 4% aqueous carboxymethylcellulose
Batch no	:	3279/141(purity > 98%)
Dose levels	:	0, 50, 150 and 450 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 at 0, 50, 150 and 450 mg/kg bw/day by gavage on days 6 to 15 after mating. The dams were observed daily for clinical signs, mortality and body weight. Food consumption was recorded on days 0, 6, 11, 16 and 21. The dams were sacrificed on day 21 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 end point).

Results
No deaths or abortions occurred at any dose level. No clinical signs were reported except for red coloration of the urine of all treated dams from day 6 to 16. Food consumption and body weight gain were reduced initially in the group treated at 450mg/kg bw/day(day 6-11). There was a compensatory increase from days 16-21 after cessation of dosing. At autopsy, a number of animals from all dose groups were found to have white intestinal worms. No other abnormalities were observed.

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 small number of foetal malformations were observed, which were within the normal range and treated groups did not differ significantly from control.
The test substance elicited maternal toxicity at the highest dose level tested but was not embryo-toxic or teratogenic. The NOAEL for materno-toxicity was considered to be 150 mg/kg bw/day. The NOAEL for the foetal organism was considered to be 450 mg/kg bw/day.

Ref. : 17

2.7. Toxicokinetics (incl. Percutaneous Absorption)

2.7.1. Percutaneous absorption *in vitro*

Study with rat skin without developer

Guideline	:	/
Tissue	:	Wistar Albino rat SPF-Cpb strain -excised skin
Method	:	Static diffusion cell
Test substance	:	Ro 1082 at 1.4% in a hair dye formulation
Batch no	:	2495/143 (purity > 98%)
Dose levels	:	0.252 mg/cm^2
Replicate cells	:	4
GLP	:	not in compliance

The skin penetration of COLIPA B87 was evaluated in a static diffusion cell using excised full-thickness rat skin. The dye was formulated in a cream at 1.36% and 18.6 mg/cm^2 of formulation was applied to 5 cm^2 of skin. It was left in place for 20 hours and then the amount of dye penetrating into the receptor fluid (physiological saline) was measured photo-metrically.

Results
The mean percutaneous penetration was reported to be 3.18%. Recovery was not calculated and use of physiological saline as receptor fluid may be inappropriate depending on the water solubility. The study is considered inadequate (see SCCNFP note of guidance).

Ref. : 9

Study with rat and pig skin, with and without developer

Guideline	:	/
Tissues	:	Wistar Albino rat strain excised skin Male pig skin, dermatomed
Method	:	Dynamic diffusion cell
Test substance	:	^{14}C-labelled Ro 1082 at 2.0% in a formulation with and without 6% H_2O_2
Batch no	:	040H9210 (radiochemical purity > 98%)
Dose levels	:	18.2 mg/cm^2 (rat) 16.9 mg/cm^2 (pig)
Treatment period	:	30 min.
Replicate cells	:	6
GLP	:	not in compliance

The radiolabelled substance was prepared at a final concentration of 2.0% in a formulation without hydrogen peroxide and 2.28% with hydrogen peroxide. Approximately 100 mg of the formulations were applied to pieces of full thickness rat skin or dermatomed pig skin fixed in diffusion cells. The formulations were rinsed off after 30 min contact with a measured volume of a standard shampoo. The amount of radioactivity penetrating through the skin into the receptor fluid (physiological saline) was measured over a period of 22 hours.

Results
The mean percutaneous penetration without hydrogen peroxide was reported to be 1.86% for rat skin and 0.56% for pig skin. The mean penetration from the formulation with hydrogen peroxide was reported to be 1.17% for rat skin and 0.09% for pig skin.
Recovery was not calculated and use of physiological saline as receptor fluid may be inappropriate depending on the water solubility.
The study is considered inadequate (see SCCNFP Notes of guidance).

Ref. : 10

2.7.2. Percutaneous absorption *in vivo*

Rat study with formulation and hydrogen peroxide

Guideline	:	/
Species/strain	:	Sprague Dawley rat, Him: OFA (SPF) strain
Group size	:	6 females
Test substance	:	^{14}C-labelled Ro 1082 at 1.85% in a hair dye formulation with H_2O_2
Batch no	:	040H9210 (radiochemical purity > 98%)
Dose levels	:	0.45 mg/cm^2
Treatment period	:	30 min under semi- occlusive conditions
Duration	:	72 hours
GLP	:	in compliance

The radiolabelled substance was prepared at a concentration of 4.0% in a hair dye formulation containing p-toluylendiamine-sulphate and immediately before used was mixed with a developer containing 6% hydrogen peroxide. 0.205g of the complete formulation, containing 4.02 mg of the test substance, was applied to 9cm^2 of intact skin of 6 female rats, that had been clipped 24 hours previously. The area of 9cm^2 was calculated to correspond to a proportion of the rat's total skin equivalent to the scalp area as a proportion of total human skin area. The formulation was left for 30 min and then scraped off with a spatula and the skin washed until free of colour. The application site was covered to prevent licking. Urine and faeces were collected daily for 72 hours, at which time the animals were sacrificed and adrenals, blood, brain, fat, femurs, heart, kidneys, liver, lungs, muscles, ovaries, spleen, thyroids, untreated skin and carcass were analysed for radioactivity. The hair-stubs were shaved off and the stratum corneum removed by tape-stripping from the application site, prior to excision of the "dermis". These three fractions were separately analysed for radioactivity.

Results
The average recovery of radioactivity was 94.5%. The mean percutaneous penetration was reported to be 0.19%, which included the amount excreted (0.18% of dose) and residual activity in the carcass (0.008% of dose). In addition, 0.041% of dose was found in the "dermis" layer of the application site, which is also potentially available to the systemic circulation. 0.22% was

found in the stratum corneum and 1.89% in the hair stubs. These fractions are not considered to be available to the systemic circulation.
Excretion was predominantly via the urine (59% of eliminated amount), and mainly in the first 24 hours (73% of total excreted radioactivity). Levels of radioactivity in the analysed organs and tissues were near or below the limits of detection.
The absorption was calculated to be 0.19%. Since the amount in the dermal layer of the application site could also be absorbed into the circulation, this should be included to produce a total absorption of 0.23%.

Ref. : 11

Rat study with integrated hair dye formulation

Guideline	:	/
Species/strain	:	Sprague Dawley rat, Him: OFA (SPF) strain
Group size	:	6 females
Test substance	:	^{14}C-labelled Ro 1082 at 1.85% in a hair dye formulation
Batch no	:	040H9210 (radiochemical purity >98%)
Dose levels	:	0.89 mg/cm^2
Treatment period	:	30 min under semi- occlusive conditions
Duration	:	72 hours
GLP	:	in compliance

The radiolabelled substance was prepared at a concentration of 4.0% in a hair dye formulation, adjusted to pH 9.5. 0.200g of the complete formulation, containing 7.98mg of the test substance, was applied to 9cm^2 of intact skin of 6 female rats, that had been clipped 24 hours previously. The area of 9cm^2 was calculated to correspond to a proportion of the rat's total skin equivalent to the scalp area as a proportion of total human skin area. The formulation was left for 30 min and then scraped off with a spatula and the skin washed until free of colour. The application site was covered to prevent licking. Urine and faeces were collected daily for 72 hours, at which time the animals were sacrificed and adrenals, blood, brain, fat, femurs, heart, kidneys, liver, lungs, muscles, ovaries, spleen, thyroids, untreated skin and carcass were analysed for radioactivity. The hair-stubs were shaved off and the stratum corneum removed by tape-stripping from the application site, prior to excision of the "dermis". These three fractions were separately analysed for radioactivity.

Results
The average recovery of radioactivity was 95.7%. The mean percutaneous penetration was reported to be 0.12%, which included the amount excreted (0.12%) and residual activity in the carcass (0.005%). In addition, 0.02% was found in the "dermis" layer of the application site, which is also potentially available to the systemic circulation. 0.16% was found in the stratum corneum and 1.40% in the hair stubs. These fractions are not considered to be available to the systemic circulation.
Excretion was predominantly via the urine (52% of eliminated amount), and mainly in the first 24 hours (70% of total excreted radioactivity). Levels of radioactivity in the analysed organs and tissues were near or below the limits of detection.
The absorption was calculated to be 0.12%. Since the amount in the dermal layer of the application site could also be absorbed in the circulation, this should be included to produce a total absorption of 0.14%.

Ref. : 12

2.7.3. Intestinal absorption *in vivo*

Guideline	:	/
Species/strain	:	Sprague Dawley rat, Him: OFA (SPF) strain
Group size	:	6 females
Test substance	:	^{14}C-labelled Ro 1082 at 1.27% in DMSO-water (7:3)
Batch no	:	040H9210 (radiochemical purity >98%)
Dose levels	:	19.9 mg/kg, by gavage
Treatment period	:	72h
GLP	:	in compliance

The radiolabelled substance was administered by gavage at 19.9 mg/kg bw to 6 female rats that had been fasted overnight. Urine and faeces were collected daily for 72 hours, at which time the animals were sacrificed and adrenals, blood, brain, fat, femurs, heart, kidneys, liver, lungs, muscles, ovaries, spleen, thyroids, skin and carcass (excluding gastrointestinal tract) were analysed for radioactivity.

Results
The average recovery of radioactivity was 98.5%. 37.7% of the dose was excreted in the urine, 93.2% of which was within the first 24 hours. 0.041% of dose was found in the carcass and 60.8% in the faeces. Radioactivity was detectable in all analysed organs and tissues, and was highest in the liver, kidneys and blood. The mean minimum oral absorption was 37.7%, i.e. the amount found in urine within the first 24 hours.

Ref. : 13

2.8. Mutagenicity/Genotoxicity

2.8.1. Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline	:	OECD 471(1983)
Species/strain	:	*Salmonella typhimurium,* TA98, TA100, TA1535, TA1537, TA1538
Replicates	:	Triplicate plates, 2 tests, conducted 4 years apart
Test substance	:	Ro 1082 dissolved in DMSO
Batch no	:	2495/64 (purity not stated in study report) Test 1 3962/46 (purity 97.9%) Test 2
Concentrations	:	4-2500 µg/plate with and without metabolic activation - Test 1 8-2500 µg/plate with and without metabolic activation - Test 2
GLP	:	in compliance

COLIPA B87 has been investigated for gene mutation in *Salmonella typhimurium* using the plate incorporation method. The first assay was conducted in 1986 and the second one in 1990. The 2 experiments were performed with different batches , the stability of the test agent having been described to be of 2 years. Liver S9 fraction from Sprague Dawley in the first experiment and Wistar liver in the second experiment rats pre-treated with Aroclor 1254 was used as the exogenous metabolic activation system. Negative and positive controls were in accordance with the OECD guideline.

Results
The test substance induced increased numbers of revertants in TA 1537, TA1538 and TA98 in the first experiment and in all tester strains in the second experiment without metabolic activation. In the presence of metabolic activation increased revertants were seen in both tests in TA98, TA100, TA1537, TA1538. The negative and positive control agents gave the expected results.

Conclusions
Based on the reversion rate, it is concluded that the test agent B 87 shows clear evidence of mutagenic activity (many folds increases over the control) in this bacterial test system in the presence or in the absence of activation system.

Ref. : 14

In Vitro Mammalian Cell Gene Mutation Test

Guideline	:	OECD 476 (1984)
Species/strain	:	Chinese Hamster V 79 cells/ HPRT Locus
Replicates	:	2 independent tests with and without metabolic activation
Test substance	:	Ro 1082 in DMSO
Batch no	:	2495/195 (purity: 99.03% with 0.07% fluorinated compounds)
Treatment time:	:	4 hours
Concentrations	:	10-100 μg/ml with and without metabolic activation
GLP	:	in compliance

The test substance was tested at concentrations of 10-100 μg/ml with and without metabolic activation. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. The concentration range was determined on the basis of maximum solubility. Negative and positive controls were in accordance with the OECD guideline. Cultures were treated for 4 hours, then incubated for 7 days before plating for 6-thioguanine resistance.

Results
The compound showed positive effects in the absence of S9 (4 doses) in the first experiment but not in the second one. According to the authors, no statistically or biologically significant increase in mutant frequency was observed over the concurrent solvent controls because of the absence of reproducibility and because the positive values observed fell within the historical control values.

Conclusions
Based on the mutation frequency rate observed in the first experiment but not in the second, the results and the study are considered equivocal.

Ref. : 15

In Vitro Mammalian Chromosome Aberration Test

Guideline	:	OECD 473 (1983)
Species/strain	:	Chinese Hamster V79 cells
Replicates	:	Duplicate cultures, 2 independent tests

Test substance	:	Ro 1082 in aqueous suspension
Batch no	:	3279/114 (purity > 95%)
Concentrations	:	10-100µg/ml without and without metabolic activation.
GLP	:	in compliance

COLIPA B87 has been investigated for induction of chromosomal aberrations in Chinese Hamster V79 cells with a 4-hour exposure time and 7, 18 and 28 hour harvest times. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system. The test concentrations were determined by the limits of solubility. Negative and positive controls were in accordance with the OECD guideline.

Results
Mitotic indices were reduced at the highest dose at 7 hours with metabolic activation and at both 18 and 28 hours without metabolic activation. The negative and positive control agents produced the expected results. No statistically and biologically significant increase in the number of cells displaying aberrations was observed with or without activation system.

Conclusions
COLIPA B 87 (or one of its metabolites) should be considered as devoid of clastogenic activity in the conditions of this test.

Ref. : 16

General Conclusions

B 87 has been tested in procaryotic cells for gene mutation in several tester strains. The results of the bacterial gene study have clearly demonstrated mutagenic properties in bacteria at the gene level. The *in vitro* test for mammalian gene mutation assay is negative, but the results are equivocal. The *in vitro* test for clastogenicity in Chinese Hamster V 79 cells is considered negative.

No conclusions can be drawn for mutagenicity.

2.9. Carcinogenicity

No data

2.10. Special investigations

No data

2.11. Safety evaluation

2.11.1. Safety evaluation, oxidative/permanent

NOT APPLICABLE

CALCULATION OF THE MARGIN OF SAFETY
(Oxidative / permanent)

The maximum concentration of … is mixed before use with H_2O_2. Thus the usage volume of 100 ml contains 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	=

2.11.2. Safety evaluation, semi-permanent

NOT APPLICABLE

CALCULATION OF THE MARGIN OF SAFETY
(Semi-permanent)

Based on a usage volume of 35 ml, containing at maximum … % (semi-permanent)

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

There is insufficient information on purity, stability and solubility.

The substance has not been adequately tested. Mutagenic potential has been shown in a bacterial assay, but no *in vivo* studies have been conducted to confirm whether this *in vitro* potential is expressed *in vivo*. It is therefore not possible to assume that a safe level of this substance can be defined on the basis of available information.

The substance has shown evidence of sensitising potential.

Severe and persistent eye irritation resulted from instillation of the neat substance. There has been no testing with a concentration relevant to use.

A 13-week oral rat study show few signs of systemic toxicity up to a dose of 180 mg/kg bw/day, the maximum dose tested. An increase in thrombocytes in the high dose group indicate that the NOAEL should be viewed as 60 mg/kg bw/day. Clarification is required with respect to the relevance of this effect and the value of NOAEL established from the 90 day oral toxicity study.

A number of studies have been reported relating to percutaneous penetration. The studies are considered inadequate.

2.13. References

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9. Henkel KGaA, Germany, Report No. RT 920194 (May 1992), citing internal report 870346, of 12/08/87
10. Henkel KGaA, Germany, Report No. TBD 910111 (July 1992)
11. Österreichisches Forschungszentrum Seibersdorf, Austria. Report No. OEFZS-A-1862 (June 1990).
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13. Österreichisches Forschungszentrum Seibersdorf, Austria. Report No. OEFZS-A-1864 (Nov 1990).
14. Henkel KGaA, Germany, Report No TBD 900613 (Oct 1990)
15. CCR, Germany, Project 122400; Henkel KGaA, Germany. Report No. TBD 880429 (6/89)
16. CCR, Germany, Project 148307; Henkel KgaA, Germany, Report No. EX 0193 (9/95)
17. RCC, Switzerland, Project 238386; Henkel KGaA, Germany, Report No EX 0406 (1990)

3. Opinion of the SCCNFP

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

Before any further consideration, the following information is required :

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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