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

CONCERNING

5-AMINO-4-CHLORO-O-CRESOL HCL

COLIPA n° A117

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 5-Amino-4-chloro-2-methylphenol hydrochloride 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

5-Amino-4-chloro-o-cresol HCl (INCI name)

2.1.2. Chemical names

Chemical name : 5-Amino-4-chloro-2-methylphenol hydrochloride
CAS name : 5-Amino-4-chloro-2-methylphenol hydrochloride
Synonyms : 2-methyl-4-chlor-5-aminophenol-hydrochlorid

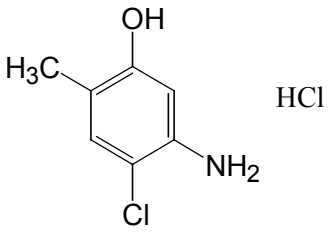
2.1.3. Trade names and abbreviations

Trade name : Ro 934, Ro 1174 (free base)
COLIPA n° : A117

2.1.4. CAS n° / EINECS n°

CAS no : 110102-85-7
110102-86-8 (free base)
EINECS n° : /

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_7H_8ClNO.HCl$
Mol weight : 157.59 (free base)
194.05 (chloride)

2.1.7. Purity, composition and substance codes

Purity : 97% (as determined by HPLC)

Impurities : 2-Methyl-5-aminophenol (dechlorinated A 117) is the main impurity up to 2 % (as determined by HPLC)

2.1.8. Physical properties

Subst. Code	:	COLIPA A117
Appearance	:	Brown crystals
Melting point	:	248 °C
Boiling point	:	no information
Density	:	no information
Rel. vap. dens.	:	no information
Vapour Press.	:	no information
Log P_{ow}	:	no information

2.1.9. Solubility

The hydrochloride is “soluble in water”, the free base is soluble in 1,2-propylene glycol and in triethanolamine.

General comments on analytical and physico-chemical characterisation

* Information on impurities is inadequate;
* Log Pow and density are not given;
* No quantitative data given for solubility.

2.2. Function and uses

COLIPA A117 is used as a hair colorant requiring the presence of hydrogen peroxide as an oxidant. It will be incorporated in hair dye formulations at a maximum concentration of 2%, for use in a 1:1 mixture with hydrogen peroxide preparation. The concentration on application is therefore 1%. It is intended for once monthly use with typical application of 100ml.

TOXICOLOGICAL CHARACTERISATION

Remark : unless otherwise stated, all toxicological tests were carried out with the hydrochloride.

2.3. Toxicity

2.3.1. Acute oral toxicity

Guideline	:	/
Species/strain	:	WA Rat
Group size	:	2 male + 2 female
Test substance	:	Ro 934 in aqueous solution
Batch no	:	2257/185 (purity not stated in study report)
Dose	:	1184, 1539 and 2000 mg/kg bw in a volume of 10 ml/kg

Observ. Period : 14 days
GLP : in compliance

Groups of 2 male and 2 female rats received a single dose of test substance by gastric gavage. The animals were observed daily for 14 days. Bodyweights were recorded weekly and macroscopic abnormalities were recorded at autopsy. No histological examinations were performed.

Results
There was one mortality, in a male rat administered the test substance at 2000 mg/kg bw; lung emphysema was noted at autopsy. The study authors concluded an LD50 for both sexes of between 1184 and 2000 mg/kg bw. The COLIPA summary interpreted the data as indicating an LD50 between 1539 and 2000 mg/kg bw for male rats, and > 2000 mg/kg bw for female rats. Clinical signs were reported in some animals of all dose groups, and included: abdominal position, apathy, piloerection, cyanosis, tremor, crouch, diarrhoea, semi-closed eyes and reduced acoustic reaction. There were no macroscopic abnormalities in 7 of the 12 rats at autopsy. The remaining animals exhibited changes such as brightened coloration of the liver and kidneys, hydrometra, ulcerations in the glandular region of the stomach and ileum, and coloured hydrocele in the intestine.

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 : /
Species/strain : Sprague-Dawley rat, CD strain (SPF)
Group size : 10 male + 10 female (15 male + 15 female for control and high dose)
Test substance : Ro 934 in aqueous solution
Batch no : 2395/56 (purity not stated in study report)
Dose levels : 0, 20, 60 and 180 mg/kg bw/day, 5 days/week by gavage

Exposure period : 13 weeks
Recovery period : 4 weeks (5 males + 5 females from control and high dose groups)
GLP : in compliance

The study report does not specifically mention OECD guideline 408, but appears to conform to its major requirements.
Groups of 15 male and 15 female rats were dosed with the test substance by gavage at 0, 20, 60 and 180 mg/kg bw/day, 5 days/week for 90 days. After 13 weeks treatment, 5 rats of each sex from the control and high dose groups were kept for 4 weeks without treatment in order to observe for potential recovery. During the study, the animals were observed for clinical signs and mortality, bodyweight and food and water consumption. At week 6, the animals were subjected to orbital bleeding for haematology and blood biochemistry analyses. These analyses were repeated at the end of the treatment and recovery periods, and 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 full treatment period.

Results
No treatment related mortalities or clinical signs of toxicity were reported. Bodyweight gain and food and water consumption were similar for all dose groups. Haematological and biochemical parameters showed some individual minor statistical differences, but these were not dose related and were not considered to be of toxicological consequence. Similarly, occasional minor abnormalities were reported at autopsy and histopathological examination for some animals of each dose group, but these were not treatment-related. No treatment related effects were reported in the recovery groups. The NOAEL was 180 mg/kg bw/day.
The rationale for selection of the dose groups is not clear. The study failed to identify a critical effect.

Ref. : 11

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 application study with substance at 10%

Guideline : OECD 404 (1981)
Species/strain : New Zealand albino rabbit (Chbb:HM)

Group size	:	5 male
Test substance	:	Ro 934 as 10% aqueous solution, adjusted to pH 8 with ammonia (see remark at end of point 2.5)
Batch no	:	(no information and purity not stated in study report)
Dose	:	0.5 ml, single dose
GLP	:	in compliance

0.5 ml of 10% aqueous test substance was applied to 6.25 cm^2 of intact skin of 5 male rabbits. Occlusive patches were applied and left in place for 4 hours. Remaining test substance was rinsed off. The skin was examined for erythema, eschar formation and oedema at 1, 24 and 48 hours after removal of the patches.

Results
No reactions were observed and the diluted substance was reported to be non-irritating to rabbit skin.

Ref. : 5

Repeat application study with substance at 10%

Guideline	:	/
Species/strain	:	Hairless mice (hr/hr)
Group size	:	5 male
Test substance	:	Ro 934 as 10% aqueous solution, adjusted to pH 8 with ammonia (see remark at end of point 2.5)
Batch no	:	(no information and purity not stated in study report)
Dose	:	1-2 drops once daily during week one and twice daily during week two
GLP	:	in compliance

One to two drops of 10% aqueous test substance were applied to a small area of skin of each animal once daily (first week) and twice daily (second week), for a total of nine consecutive working days. The skin was examined before each application for erythema, eschar formation and oedema.

Results
No primary skin reactions were observed and the diluted substance was reported to be non-irritating to mouse skin. The study report is insufficient to establish details of the experimental protocol and does not include any data.

Ref. : 6

Single application study with neat substance

Guideline	:	OECD 404 (1981)
Species/strain	:	New Zealand albino rabbit (SPF)
Group size	:	3 female
Test substance	:	Ro 934
Batch no	:	3279/64 (purity > 99%)
Dose	:	0.5 g of pure test substance moistened with RO (reverse osmosis) water
GLP	:	in compliance

0.5 g of moistened test substance was applied to 6 cm^2 of intact skin of 3 female rabbits. Semi-occlusive patches were applied and left in place for 4 hours. Remaining test substance was removed by swabbing with cotton wool swabs soaked in tap water. The skin was examined for erythema, eschar formation and oedema at 1, 24, 48 and 72 hours after removal of the patches.

Results
Very slight erythema was observed on two of the rabbits and very slight oedema on the third after 1 hour. These reactions resolved within 24 hours. Brown/yellowish staining due to the test substance was reported after 1 hour, reducing to very light yellow by 72 hours. According to the defined criteria, the test substance was reported to be non-irritating to rabbit skin.

Ref. : 4

2.4.2. Irritation (mucous membranes)

Irritancy of 5% solution

Guideline	:	OECD 405
Species/strain	:	New Zealand albino rabbit, (Chbb:HM)
Group size	:	4 male
Test substance	:	Ro 934, 5% aqueous solution, adjusted to pH8 with ammonia (see remark at end of point 2.5)
Batch no	:	(no information and purity not stated in study report)
Dose	:	0.1 ml
GLP	:	in compliance

0.1 ml of the test substance was applied once to the right eye of 4 male rabbits, without rinsing. The left eye served as control. Ocular reactions were recorded at 1, 6, 24 and 48 hours after instillation.

Results
Slight redness of the conjunctiva was reported in 3 rabbits within 6 hours of instillation, which had resolved in all cases by 24 hours. In addition, exudate was observed in all rabbits one hour after instillation, in 3 after 6 hours and remaining in one animal until 24 hours. No reactions were reported in the cornea or iris. The test substance was reported to be slightly irritant to the rabbit eye.

Ref. : 2

Irritancy of neat substance

Guideline	:	OECD 405
Species/strain	:	New Zealand albino rabbit, SPF quality
Group size	:	1 female
Test substance	:	Ro 934, neat
Batch no	:	3279/64 (purity >99%)
Dose	:	51 mg
GLP	:	in compliance

51 mg of the pure test substance was applied to the right eye of one female rabbit, without rinsing. The left eye served as control. Ocular reactions were recorded at 1 hour and 1, 2, 3 and 7 days after instillation.

Results
The test substance caused an immediate severe reaction and therefore was not administered to additional animals. Reactions included opacity of the cornea, injection of the iris and irritation of the conjunctivae, where were irreversible within the 7 day study period. There was evidence of ocular corrosion and investigation with fluorescein revealed corneal epithelial damage. The substance was extremely irritating under the test conditions.

Ref. : 3

2.5. Sensitisation

Magnusson and Kligman study

Guideline	:	/
Species/strain	:	Pirbright White guinea pig
Group size	:	20 test + 20 control, female
Test substance	:	Ro 934, aqueous solutions, adjusted to pH8 with ammonia (see remark at end of point 2.5)
Batch no	:	2395/56 (purity not stated in study report)
Concentrations	:	intradermal induction : 0.1 ml Freund's complete adjuvant (FCA) 0.1 ml 0.25% test substance 0.1 ml 0.5% test substance/FCA (1:1) topical induction : 1 ml 5% test substance for 48 hours, occluded challenge : 0.2 ml 2% test substance for 24 hours, occluded
GLP	:	in compliance

The protocol was an adaptation of the Magnusson and Kligman method and appears to conform to OECD guideline 406, which is not cited in the study report.
Induction commenced with three intradermal injections, of FCA, test substance (0.25%), and a mixture of these two. One week later the induction process was completed with a single topical application of 1ml of the test substance (5%) under occlusive patch for 48 hours. 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%) under occlusive patch on the flank for 24 hours. Appropriate controls were treated with vehicle at all stages. The skin was examined 24 and 48 hours after removal of the challenge patches.

Results
Twenty four hours after the challenge, 10 of the 20 test animals showed slight erythema, accompanied by oedema in one animal. Reactions were seen in 9 of the 20 animals, of which two had oedema, after 48 hours. No reactions were observed in control animals. The substance was a moderate sensitiser under the test conditions.

Ref. : 7

Magnusson and Kligman study with formulation containing p-toluidine diamine

Guideline	:	/	
Species/strain	:	Pirbright White guinea pig	
Group size	:	20 test + 20 control, female	
Test substance	:	Ro 934 in formulation containing p-toluidine diamine, diluted 1:1 with 6% H_2O_2 (Ro 934/H_2O_2 mix)	
Batch no	:	Batch 2395/56 (purity not stated in study report)	
Concentrations	:	intradermal induction :	0.1 ml FCA 0.1 ml 0.1% test substance in Ro934/H_2O_2 mix 0.1 ml 0.2% test substance in Ro934/H_2O_2 mix/FCA (1:1)
		topical induction :	1 ml 10% aqueous test substance for 48 hours, occluded
		challenge :	0.2 ml 2.5% test substance in Ro934/H_2O_2 mix for 24 hours, occluded
GLP	:	in compliance	

The protocol was an adaptation of the Magnusson and Kligman method and appears to conform to OECD guideline 406, which is not cited in the study report.
Induction commenced with three intradermal injections, of FCA, test substance (0.1% in peroxide-containing formulation with p-toluidine diamine), and a mixture of these two. One week later the induction process was completed with a single topical application of 1ml of the test substance (10%, aqueous) under occlusive patch for 48 hours. 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% in peroxide-containing formulation) under occlusive patch on the flank for 24 hours. Appropriate controls were treated with vehicle at all stages. The skin was examined 24 and 48 hours after removal of the challenge patches.

Results
No reactions were observed in control or test animals after removal of the patches. The formulation containing the test substance was non-sensitising under the test conditions.

Ref. : 8

Magnusson and Kligman study with formulation containing 2,4,5,6-tetraamino-pyrimidine

Guideline	:	/	
Species/strain	:	Pirbright White guinea pig	
Group size	:	20 test + 20 control female	
Test substance	:	Ro 934 in formulation containing 2,4,5,6-tetraamino-pyrimidine, diluted 1:1 with 6% H_2O_2 (Ro 934/H_2O_2 mix)	
Batch no	:	2395/56 (purity not stated in study report)	
Concentrations	:	intradermal induction:	0.1 ml FCA 0.1 ml 0.1% test substance in Ro934/H_2O_2 mix 0.1 ml 0.2% test substance in Ro934/H_2O_2 mix/FCA (1:1)
		topical induction :	1 ml 20% aqueous test substance for 48 hours, occluded

		challenge :	0.2 ml 2.5% test substance in Ro934/H_2O_2 mix for 24 hours, occluded
GLP	:	in compliance	

The protocol was an adaptation of the Magnusson and Kligman method and appears to conform to OECD guideline 406, which is not cited in the study report.
Induction commenced with three intradermal injections, of FCA, test substance (0.1% in peroxide-containing formulation with 2,4,5,6-tetraamino-pyrimidine), and a mixture of these two. One week later the induction process was completed with a single topical application of 1ml of the test substance (20%, aqueous) under occlusive patch for 48 hours. 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% in peroxide-containing formulation) under occlusive patch on the flank for 24 hours. Appropriate controls were treated with vehicle at all stages. The skin was examined 24 and 48 hours after removal of the challenge patches.

Results
No reactions were observed in control or test animals after removal of the patches. The formulation containing the test substance was non-sensitising under the test conditions.

Ref. : 9

Buehler study on free base

Guideline	:	OECD 406	
Species/strain	:	Dunkin-Hartley albino guinea pig	
Group size	:	10 male + 10 female, control + test	
Test substance	:	Ro 1174 – 5-amino-4-chloro-2-methylphenol, free base, suspended in ethanol	
Batch no	:	3729/143 (purity not stated in study report)	
Concentrations	:	topical induction :	3 x 0.5 ml 63% test substance paste in ethanol
		challenge:	0.1-0.2 ml 10% test substance for 24 hours
GLP	:	in compliance	

Topical induction was by three 0.5 ml applications of test substance (63% as paste in ethanol), for 6 hours under occluded patch to the left flank on three occasions (days 1, 8 and 15). An interval of 2 weeks was allowed after induction and then the animals were challenged by a single 0.5 ml topical application of the test substance (63% as paste in ethanol) under occlusive patch on the right flank for 6 hours. Appropriate controls were treated with vehicle at all stages. The skin was examined 24 and 48 hours after removal of the challenge patches.

Results
The test material did not induce a reaction in any of the treated animals. Irritation was observed in some control animals and was considered to be due to an interaction between the vehicle and shaving.

Ref. : 10

Remark :

Point 2.4. Eye irritation

The eye irritation test with the neat compound showed severe reactions. Tests with a 5 % aqueous solution of A 117, with an adjusted pH 8.0, have been well tolerated. This is a more realistic scenario to mimic application conditions, intended for final formulations.

Point 2.5. Sensitisation

The hair dye A 117 was tested in both configurations as hydrochloride (Ro 934) as well as free base (Ro 1174). Besides the impact on acidity, water solubility is enormously influenced by changing the pH of A 117, the hydrochloride is freely soluble, the free base is only suspendable in water. To avoid acidity based incompatibilities of the hydrochloride, the pH value was adjusted to pH 8 using ammonium hydroxide. Under such pH conditions the local compatibility is acceptable and the development of purely soluble aminochloro methylphenol salts is only marginal. Moreover the described procedure is mimicking realistic conditions of the hair dyeing process.

2.6. Teratogenicity

Guideline	:	OECD 414 (1981)
Species/strain	:	Wistar/HAN rat, Kfm: WIST, outbred SPF strain
Group size	:	25 females (mated)
Test substance	:	Ro 934 in aqueous solution
Batch no	:	3279/89 (purity c. 98%)
Dose levels	:	0, 20, 100 and 500 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 on days 6 to 15 after mating. Dose levels were set at 0, 20, 100 and 500 mg/kg bw/day. The dams were observed at least twice daily for clinical signs and mortality, bodyweight was recorded daily and food consumption on days 6, 11, 16 and 21. They 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 endpoint).

Results
There were no treatment-related mortalities and no clinical signs of reaction to the test substance except for yellow-brown discoloration of the urine in the dams at all dose levels. A decrease in food consumption was noted in the dams receiving 500 mg/kg bw over the period from days 6 to 11 (reduction of 4.9% compared with controls), but there were no differences in body weight gain and so this was not considered to be treatment-related.

No macroscopic abnormalities were observed in any of the dams at autopsy. The mean numbers of corpora lutea, implantation sites, post-implantation loss, live foetuses and the mean foetal bodyweights were similar for control and treated groups. There were no macroscopic anomalies

or malformations in any of the foetuses of the control or high dose groups. Abnormal findings in a very small number of foetuses of the two lower dose groups at the macroscopic level, and in the controls and 20 and 500 mg/kg bw/day dose groups at the microscopic level were considered to be within the normal range and not of toxicological relevance.
The test substance had no effect on maternal or foetal parameters and was not embryo-toxic or teratogenic up to and including the dose of 500 mg/kg bw/day.

Ref. : 18

2.7. Toxicokinetics (incl. Percutaneous Absorption)

2.7.1. Percutaneous absorption *in vivo*

Rat *in vivo* study with formulation

Guideline	:	/
Species/strain	:	Sprague Dawley rat, Him: OFA (SPF) strain
Group size	:	6 females
Test substance	:	Methyl-^{14}C-labelled Ro 934 at 1.85% in a hair dye formulation
Batch no	:	2534-150 (radiochemical purity >99%)
Dose levels	:	0.41 mg/cm^2
Treatment period	:	72h under semi-occlusive patch
GLP	:	in compliance

The radiolabelled substance was prepared at a final concentration of 1.85% in a hair dye formulation containing p-toluylendiamine-sulphate. Hydrogen peroxide was not included. 0.201g of the formulation, containing 3.71 mg of the test substance, was applied to 9 cm^2 of intact skin of 6 female rats, that had been clipped 24 hours previously. The area of 9 cm^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. A semi-occlusive patch was applied to the site and left in place for 72 hours, during which urine and faeces were collected daily. Residual formulation on the skin surface was washed off until the skin was free of colour, and analysed for radioactivity, as were the patches. 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 93.4%. The mean percutaneous penetration was reported to be 32.7%, which included the amount excreted (32.6%) and residual activity in the carcass (0.12%). In addition, 0.14% 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 6.57% in the hair stubs. These fractions are not considered to be available to the systemic circulation.

Excretion was predominantly via the urine (92% of total excreted radioactivity), and mainly in the first 24 hours (78% of total excreted radioactivity). Radioactivity was detected in all of the analysed organs and tissues, with the highest content found in the kidneys.
The study authors and COLIPA summary concluded absorption of 32.7%. 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 32.86%. The exposure of 72 hours under semi-occlusive patch is likely to overestimate penetration under conditions of use.

Ref. : 12

Rat *in vivo* study with formulation and hydrogen peroxide

Guideline	:	/
Species/strain	:	Sprague Dawley rat, Him: OFA (SPF) strain
Group size	:	6 females
Test substance	:	Methyl-^{14}C-labelled Ro 934 at 1.85% in a hair dye formulation with H_2O_2
Batch no	:	2534-150 (radiochemical purity >99%)
Dose levels	:	0.41 mg/cm^2
Treatment period	:	30 min.
GLP	:	in compliance

The radiolabelled substance was prepared at a concentration of 3.7% in a hair dye formulation containing p-toluylendiamine-sulphate and immediately before used was mixed with a developer containing 6% hydrogen peroxide (final concentration is 1.95 %). 0.200g of the complete formulation, containing 3.66 mg of the test substance, was applied to 9cm^2 of intact skin of 6 female rats, that had been clipped 24 hours previously (0.41 mg/cm²). 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 96.7%. The mean percutaneous penetration was reported to be 1.28%, which included the amount excreted (1.27%) and residual activity in the carcass (0.009%). In addition, 0.2% was found in the "dermis" layer of the application site, which is also potentially available to the systemic circulation. 0.2% was found in the stratum corneum and 7.63% in the hair stubs. These fractions are not considered to be available to the systemic circulation.

Excretion was predominantly via the urine (91% of total excreted radioactivity), and mainly in the first 24 hours (92% of total excreted radioactivity). Levels of radioactivity in the analysed organs and tissues were near or below the limits of detection.

The study authors and COLIPA summary concluded absorption of 1.28%. 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 1.48% (6.07 mg/cm²).

Ref. : 13

2.7.2 Intestinal absorption *in vivo*

Guideline	:	/
Species/strain	:	Sprague Dawley rat, Him: OFA (SPF) strain
Group size	:	6 females
Test substance	:	Methyl-^{14}C-labelled Ro 934 at 1.27% in propylene glycol-water (1:1)
Batch no	:	2534-150 (radiochemical purity >99%)
Dose levels	:	21.5 mg/kg, by gavage
Treatment period	:	72h
GLP	:	in compliance

The radiolabelled substance was administered by gavage at 21.5 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 97.4%. 91.7% of the dose was excreted in the urine, almost all within the first 24 hours (91.5% of dose). 0.31% of dose was found in the carcass and 5.7% in the faeces (5.59% in the first 24 hours). Radioactivity was detectable in all analysed organs and tissues except for the thyroids and muscle, and was highest in the liver and skin.

Ref. : 14

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline	:	/
Species/strain	:	*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537, TA1538
Test substance	:	Ro934 (hydrochloride) in bi-distilled water (expt 1) Ro1174 (free base) in DMSO solution (expt 2)
Batch no	:	Ro 934: lot 2257/180 (purity not stated in study report) Ro 1174: lot 3279/143 (purity not stated in study report)
Concentrations	:	4-2500 µg/plate (expt 1), 75-1200 µg/plate (expt 2), with and without metabolic activation
GLP	:	in compliance

COLIPA A117, as both the hydrochloride and the free base, has been investigated for gene mutation in *Salmonella typhimurium*. Liver S9 fraction from Aroclor 1254-induced rats was used as the exogenous metabolic activation system.

Results

First experiment (Ro 934)
– S9 : negative in all tester strains
+ S9 : slightly positive (x 1.8) with a trend dose response for TA 100.

Second experiment (Ro 1174)
+ S9 : positive in TA 100 tester strains from 150 µg/plate and upward with a trend dose response
+ : positive in TA 1538 tester strains from 300 µg/plate and upward with a trend dose response
- S9 : negative in all tester strains.

Conclusions
Based on the reversion rate, it is concluded that the test agent A 117 shows evidence of mutagenic activity in TA 100 in the presence of activation system with one substance Ro 934, and positive in TA 100 and TA 1538 in the presence of activation system with Ro 1174. Replicates were not performed with the same substance.

Ref. : 15

In Vitro Mammalian Cell Gene Mutation Test

Guideline	:	OECD 476
Species/strain	:	Chinese Hamster V79 cell line / HGPRT Locus
Replicates	:	2 independent tests with and without metabolic activation
Test substance	:	Ro 934 in ethanol
Batch no	:	2395/56 (purity not stated in the study report)
Treatment time	:	4 hours
Purity	:	purity not given
GLP	:	in compliance

Liver S9 fraction from Aroclor 1254-induced Wistar rats was used as the exogenous metabolic activation system.

Results
The compound shows negative effects both in the absence and in the presence of S9 in both experiments. According to the study authors, no biologically significant relevance was associated with the slight positive results obtained at 350 µg/ml in the presence of activation.

Conclusions
Based on the mutation frequency rate, no biologically significant relevance was associated with the positive results obtained

Ref. : 16

2.8.2 Mutagenicity/Genotoxicity *in vivo*

In Vivo Mammalian Erythrocyte Micronucleus Test

Guideline	:	OECD 474 (1983)
Species/strain	:	CFW1 mouse
Group size	:	5 male + 5 female
Test substance	:	Ro 934 in aqueous solution
Batch no	:	2395/56 (purity not stated in the study report)
Dose levels	:	50, 250 and 500 mg/kg bw, gavage
Sacrifice times	:	24, 48 and 72 hours after dosing

GLP : in compliance

COLIPA A171 has been investigated for induction of micronuclei in the bone marrow cells of CFW1 mice. The substance was administered once by gavage at 500 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
No clinical signs of toxicity were noted.

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 for the high dose. The latter having given negative results, the two lower doses were not scored.

PCE/NCE ratio
Groups of mice treated with COLIPA A117 did not exhibited a significant variation of the PCE/NCE ratio.

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, it should be noticed that the absence of variation in the PCE/NCE ratio does not allow to determine if the test agent has reached the bone marrow. Moreover, the rationale for not scoring low and intermediate dose is not usual.

Ref. : 17

General Conclusions

* A117 was tested in procaryotic cells for gene mutation in 5 tester strains . The results of the bacterial gene study demonstrated mutagenic properties at the gene level in some tester strains (TA 100 and TA 1538) in the presence of activation . However, the substances tested are not identical in the 2 experiments performed. This test does not conform to OECD guidelines and should be repeated.
* The *in vitro* test for mammalian gene mutation assay is negative.
* The *in vivo* micronucleus test in CFW1 mice gave negative results. However, it should be noticed that the absence of variation in the PCE/NCE ratio does not allow to determine if the test agent has reached the bone marrow. In addition, only the highest dose has been scored.

2.9. Carcinogenicity

No data

2.10. Special investigations

No data

2.11. Safety evaluation

NOT APPLICABLE

CALCULATION OF THE MARGIN OF SAFETY

(5-Amino-4-Chloro-o-Cresol HCl)
(Oxidative/Permanent)

The maximum concentration of … % 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	=

2.12. Conclusions

Insufficient information is provided on physico-chemical properties : solubility, potential impurities, stability. The database on toxicological properties is inadequate. Acute toxicity, irritation, sensitisation and embryotoxicity have been adequately investigated. The substance has shown sensitisation potential. The subchronic study in rats used low dose levels in comparison to the acute toxicity and failed to identify any adverse effects. However it complied with OECD guidelines and a NOAEL of 180 mg/kg bw/day can be assumed. Skin penetration has been investigated in the rat *in vivo*. Sufficient information on tissue distribution and recovery is provided to assume that the results are acceptable.

The major deficiency is in mutagenicity testing. The test for bacterial gene mutation was not in compliance with OECD guidelines, and gave a positive result in the presence of metabolic activation. Negative results were obtained for mammalian cell mutation *in vitro*.
The *in vivo* micronucleus test in CFW1 mice gave negative results. However, it should be noticed that the absence of variation in the PCE/NCE ratio does not allow to determine if the test agent has reached the bone marrow. In addition, only the highest dose has been scored.

2.13. References

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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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