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

CONCERNING

TETRAHYDRO-6-NITROQUINOXALINE

Colipa n° B104

adopted by the SCCNFP during the 19th Plenary meeting
of 27 February 2002

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 tetrahydro-6-nitroquinoxaline 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

Tetrahydro-6-nitroquinoxaline (INCI-name)

2.1.2. Synonyms

1,2,3,4-Tetrahydro-6-nitro-quinoxaline (CAS name)
1,2,3,4-Tetrahydro-6-nitro-chinoxaline
6-nitro-1,2,3,4-Tetrahydro-chinoxaline

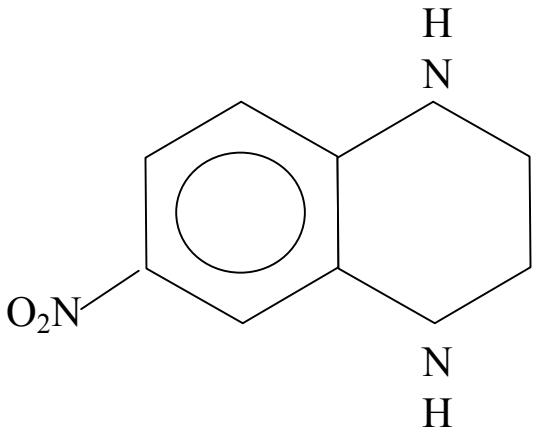
2.1.3. Trade names and abbreviations

Ro 1135 (hydrochloride)
Ro 1173 (free base)

2.1.4. CAS no.

41959-35-7 (free base)
158006-54-3 (hydrochloride)

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_8H_9N_3O_2$
Mol weight : 179.17

2.1.7. Purity, composition and substance codes

Subst. Code : Ro1135 (HCl); Ro1173 (free base)
Purity : 98-99 % (determined by HPLC)

2.1.8. Physical properties

Appearance : odourless dark red to dark brown powder (free base); brown powder (HCl)
Melting point : 113 °C (free base); 212-213 °C (HCl)
Boiling point : /
Density : /
Rel. vap. dens. : /
Vapour Press. : /
Log P_{ow} : /

2.1.9. Solubility

Poorly soluble in water, soluble in ethanol (free base)
Soluble in water (HCl)

2.2. Function and uses

B104 is used as hair dye up to a final concentration of 1.0% on head in the presence or absence of a developer-mix.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

An acute oral toxicity study of 1,2,3,4-tetrahydro-6-nitroquinoxaline hydrochloride (Ro 1135) was performed in the Wistar Rat according to the OECD guideline N°401 (1987).

The oral median acute lethal dose was estimated to 1058 mg/kg body weight (1275 mg/kg males; 860 mg/kg females).

Ref. : 1

2.3.2. Acute dermal toxicity

An acute dermal toxicity study of 1,2,3,4-tetrahydro-6-nitroquinoxaline (Ro 1173) suspended in an aqueous solution of 1 % carboxymethylcellulose was conducted in the Wistar Rat according to the OECD guideline N°402 (1987)**.**

No mortality occurred during the study period, the acute lethal dose was found to be exceeding 2000 mg/kg body weight after dermal application.

Ref. : 2

2.3.3. Repeated dose dermal toxicity

50 µl of a 3% aqueous solution of 1,2,3,4-tetrahydro-6-nitroquinoxaline hydrochloride was applied to the skin of 6 New Zealand White Rabbits once daily for 5 consecutive days per week for three weeks in total, following the method of Marzulli and Maibach.

Under the experimental conditions adopted, neither symptoms of systemic intoxication nor skin irritation were observed with the tested preparation.

Ref. : 4

2.3.4. Sub-chronic oral toxicity

A 13-week oral toxicity study was conducted in 80 Wistar rats (4 groups of 10 rats per sex) with 1,2,3,4-tetrahydro-6-nitroquinoxaline (Ro 1173) according to the OECD guideline N° 408 (1981) with 10 additionally animals allowed a 4 week recovery period after treatment**.** The test compound was administered by gavage as an aqueous suspension incorporating 1 % carboxymethylcellulose, at once daily dosages of 0, 5, 25 and 125 mg/kg body weight. No mortality was observed during the treatment period. Male rats of the high dose group showed a reduction of their body weight gain. Food consumption was not affected by the treatment. Orange discoloured urine, tinted skin and fur were noted ; these observations were dose related and considered to be due to staining of the test substance. Salivation occurred in all treated groups and lethargy was seen in the 125 mg/kg/day ; these data were considered treatment related. A yellow discoloured retina concerning 8 animals of the high dose group was observed and was also attributed by investigators to the physical properties of the test article and therefore of no toxicological relevance. A decrease in red blood cells number and an increase in γ–glutamyl transferase were also observed in both males and females of the high dose group. A persisting yellow, orange or brown staining of various organs was noted for all animals treated with 25 and 125 mg/kg/day of the test article. 3/5 females of the high dose group were with distended, water-filled uteri. The absolute liver and spleen weight in the medium and high dose group was increased but was reversed after a 4 week recovery period. Pigmentation was seen in specific organs like the liver, the kidneys, mesenteric lymph nodes and gastro-intestinal tract in the medium and high dose group and was slightly reduced after recovery period. An increased haematopoietic activity in the spleen was also noted in the male rats of the high dose group.

Based on these results, the NOEL (No-Observed-Effect-Level) was established by the investigators to be at least 5 mg/kg/day for 1,2,3,4-tetrahydro-6-nitroquinoxaline.

Ref. : 17

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Study 1

Guideline	:	OECD 404 (1981)
Species/strain	:	New Zealand albino rabbits
Group size	:	3 female
Test substance	:	Ro1135
Batch no	:	3359/100 (purity > 98%)
Dose	:	0.5 g
GLP	:	QA statement included

The substance was applied (0.5 g) to shaved dorsal skin (6 cm^2), and covered by semi-occlusive patches for 4 hours. Cutaneous reactions were evaluated 1, 24, 48 and 72 hours after removal of the patches.

Results
Orange staining was observed and a primary irritation index (PI index) of 0.17 (mildly irritating) was calculated. Slight erythema was seen in one animal, reversible within 48 hours after exposure.

Ref. : 3

Study 2

Guideline	:	Modification of guideline 404 (1981)
Species/strain	:	New Zealand albino rabbits
Group size	:	6 females
Test substance	:	Ro1135
Batch no	:	3359/100 (purity >98%)
Dose levels	:	0.05 ml of 3% (w/w) solution of the test article in water
GLP	:	QA statement included

A fresh aqueous solution was prepared daily. The dorsal fur was shaved. A volume of 0.05 ml of the formulation was applied and spread on approximately 6 cm^2 using a syringe. Applications were performed daily, 5 days pr. week for 3 weeks. The Draize reading scale was used.
The skin reactions were assessed approximately 24 hours after each application just before the next treatment. The shaved skin area around the exposed area was used as reference. The skin area was shaved repeatedly every dosing day, approximately 3 hours before observation.

Results
No skin irritation was observed during the study period. The treated skin of all animals showed an orange red discoloration by the test article. One animal was killed prior to end of the study for human reasons, not related to the exposure to the test compound.

Ref. : 4

2.4.2. Irritation (mucous membranes)

Study 1

Guideline	:	OECD guideline 405 (1987)
Species/strain	:	New Zealand albino rabbits
Group size	:	3 females
Test substance	:	Ro 1135
Batch no	:	3359/100 (purity >98%)
Dose	:	51±1 mg (equivalent to 0.1 ml)
GLP	:	QA statement included

The test substance was applied neat to the conjunctival sack of the right eye without rinsing. The left eye served as controlled and was untreated. Ocular reactions were recorded at 1, 24, 48 and 72 hours and 7, 14, and 21 days after instillation of the test article.

Results
The test article appears to be extremely irritating to the rabbit eye (Draize score 65.3). The reactions affected the cornea, iris and conjunctivae. Their opacity of the cornea was reversible within 21 days in 1 animal and irreversible in 2 other animals. The injection of the iris was reversible within 14 days in 1 animal, but couldn't be assessed in the 2 other animals. Oral staining of the cornea and the conjunctivae by the test article was observed. The test article caused corrosion on the cornea of 2 animals and great white discoloration of the eyelids as a sign of necrosis in all 3 animals.

Ref. : 5

Study 2

Guideline	:	OECD 405 (1987)
Species/strain	:	New Zealand albino rabbits
Group size	:	3 females
Test substance	:	Ro 1173, 1,2,3,4-tetrahydro-6-nitroquinoxaline
Batch no	:	3933/58 (purity±99%)
Dose	:	60±1 mg
GLP	:	QA statement included

The test substance was instilled in the conjunctival sack of the right eye. The other eye remained untreated and served as control. Ocular reactions were recorded at 1, 24, 48, 72 hours and 7 days after instillation of the test substance.

Results
Adverse effects were recorded on the cornea and conjunctivae. The opacity of the cornea was reversible within 72 hours and the irritation of the conjunctiva was reversible within 7 days. The test substance is considered mildly irritating to the rabbit eye.

Ref. : 6

2.5. Sensitisation

Magnusson & Kligman study

Guideline	:	OECD 406 (1981)	
Species/strain	:	Dunkin-Hartley guinea pig	
Group size	:	35 animals, 5 in primary irritation study, 10 animals in control group, and 20 in test group	
Test substance	:	Ro 1135, brown/crystalline solid	
Batch no	:	3359/100 (purity >98%)	
Concentration	:	Intradermal induction	0.1 ml 2.5% test substance in physiological saline 0.1 ml 50% Freund's complete adjuvant (FCA) 0.1 ml 2.5% test substance in FCA/saline emulsion (1:1)
		Topical induction	0.5 ml 25% test substance in water, occluded
		Challenge	0.05 ml of test substance in 3 concentrations 10%, 5%, 2.5% in water, occluded
GLP	:	QA statement included	

Induction was performed with 3 intradermal injections of test substance (2.5%), Freund's compete adjuvant and a mixture of these two. Topical induction was performed 7 days later by occlusive patch for 48 hours to the same nucheal area of the animals with 0.5 ml of the test substance (25%). After a rest period of 2 weeks the animals were challenged by a single closed patch to the flank with a test substance in 3 concentrations (10%, 5%, and 2.5% in water). The patches were removed after 24 hours. The control animals were treated with vehicle. The skin was examined 24 and 48 hours after removal of the bandage.

Results
19 of 20 test animals showed a positive reaction in response to the 10% and 5% concentration and 18 animals reacted positively to the 2.5% concentration. The reactions were characterised by crust formation, swelling and scaliness.

Thus the compound led to a sensitisation rate of 95%, which indicates that Ro1135 has extreme sensitising properties.

Ref. : 7

Buehler Test 1

Guideline	:	OECD 406 (1981)
Species/strain	:	Dunkin-Hartley guinea pig
Group size	:	44 guinea pigs, 2 males and 2 females in preliminary study; 10 males and 10 females in control group, and 10 males and 10 females in test group
Test substance	:	Ro 1135
Batch no	:	3359/156 (purity not stated in report)
Concentration	:	Topical induction with 0.5 ml 53% test substance in water (aqueous paste) occluded for 6 hours

		Challenge with 0.5 ml 53% test substance in water (aqueous paste) occluded for 6 hours.
GLP	:	QA statement included

Topical induction was performed on one side of the trunk on days 1, 8 and 15. Controls received the vehicle. Challenge was carried out 2 weeks later by applying 0.5 ml of the same paste under occlusion for 6 hours to untreated animal flanks.
Reading was performed after 24 and 48 hours after removal of the patches.

Results
The test sites were stained by the test substance and visual reading was not possible. Therefore biopsies were taken from the test sites for histological examination. According to the test report, eczematous skin reaction was seen in 5 out of 20 treated animals and in 3 out of 20 control animals.
The test is inclusive because microscopic evaluation of patch test reactions cannot differentiate between toxic and allergic reactions.

Ref. : 8

Buehler test 2

Guideline	:	OECD 406 (1981)
Species/strain	:	Dunkin-Hartley guinea pig
Group size	:	44 animals, 4 in preliminary test group, 10 females and 10 males in test group, 10 females and 10 males in control group
Test substance	:	Ro 1173,1,2,3,4-tetrahydro-6-nitroquinoxaline
Batch no	:	3933/58 (purity not stated in report)
Concentration	:	Topical induction with 0.5 ml 10% test substance in ethanol suspension occluded for 6 hours Challenge with 0.5 ml 10% test substance in ethanol suspension occluded for 6 hours
GLP	:	QA statement included

Induction was performed by 3 topical applications on day 1, 8, and 15. The control animals received 0.5 ml ethanol. After 2 weeks rest period the animals were challenged on day 29 with 0.5 ml of test article in a 10% ethanol suspension and occluded for 6 hours. The test sites were scored 24 and 48 hours after removal of the patches.

Results
The test sites were stained by the test substance and visual scoring was not possible.
Histopatological examination of the skin was carried out in 18 control animals and in 19 test animals. The test article provoked eczematous reaction in 1:19 test animals and in 3:18 control animals.
The study is inconclusive, because it is not possible to distinguish between irritation and sensitisation on the basis of histological examination.

Ref. : 9

2.6. Reproduction Toxicity

1,2,3,4-tetrahydro-6-nitroquinoxaline (Ro 1173) dissolved in water with 4 % carboxymethylcellulose was administered orally by gavage to pregnant female Wistar rats (25 animals per group) on day 6 through 15 of gestation at the dose levels of 10, 30 and 100 mg/kg/day body weight according to the OECD N°414 (1981). A control group was administered with the vehicle only. Pregnant animals were killed on day 21 post-coitum ; macroscopic pathological changes were examined on the dams, visceral and skeletal malformations were observed on the foetuses. No female mortality was recorded during the study. Dose related orange discoloured urine was noted in all dosed animals. A slight decrease of body weight gain and food consumption was seen during the treatment period with the medium and the high dose group. A marginal treatment related post-implantation loss was recorded in the dams of the high dose group. No specific findings were seen concerning external examination, sex ratios, mean body weights, visceral examination of the foetuses excepted a slightly delayed maturation of skeletal development at 100 mg/kg. This finding was considered by the investigators to be due to the primary maternal toxicity.

Under the experimental conditions adopted, 1,2,3,4-tetrahydro-6-nitroquinoxaline revealed none teratogenic, embryo-toxic or embryo-lethal effects, the No-Observable-Adverse-Effect-Level of the test product was established by the investigators at 10 mg/kg/day body weight for the dams and at 100 mg/kg/day for the foetuses.

Ref. : 18

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Percutaneous absorption in vivo, study 1

Guideline	:	Not available
Species/strain	:	Sprague Dawley strain rats (Him: OFA, SPF)
Test substance	:	Basic hair dye formulation excluding a developer with approximately 1% of radiolabelled RO 1173 (1,2,3,4-tetrahydro-6-nitroquinoxaline). The formulation consisted of 1,2,3,4-tetrahydro-6-nitroquinoxaline 1% in emulsifier basis Bth 66, ammonium sulphate, ammonia and water. The pH value was adjusted to 9,0.
Batch no	:	3933/177 (purity unknown)
Dose levels	:	Approximately 200 mg of the formulation/9 cm^2 rat skin
GLP	:	QA statement included

The dermal absorption/percutaneous penetration of ^{14}C labelled 1,2,3,4-tetrahydro-6-nitroquinoxaline was studied on clipped skin of 6 female rats. The animals were exposed to the test formulation for 30 minutes without occlusion and they were kept in metabolism cages. With an area of 9 cm^2 the dose was approximately 0.23 mg/cm^2. Urine and faeces were taken as fractions for 0-24 hours, 24-48, and 48-72 hours. The animals were then killed and the radioactivity was determined in different layers of the treated skin, in special organs, in the carcass and in the excretions.

Results
2.4 μg/cm^2 was percutaneously absorbed. 0.115 μg/cm^2 of the applied test substance remained in the skin after tape stripping of the stratum corneum, and is taken to be systemically available. The radioactivity was excreted mainly via urine, (about 56%) and to a lesser extent via faeces, (about 44%) with a total mass balance of >97%. Most of the radioactivity was eliminated within the first 24 hours after application (86%).

Ref. : 15

Percutaneous absorption in vivo, study 2

Guideline	:	Not available
Species/strain	:	Sprague Dawley Rat (Him: OFA, SPF)
Test substance	:	A formulation containing the test compound RO 1173 (1,2,3,4-tetrahydro-6-nitroquinoxaline) and other hair dye components, Resorcinol, 2,5-diaminotoluene sulphate, sodium sulphite, emulsifier (SR 484), ammonium sulphate, water, ammonia.
Batch no	:	Hair dye formulation 06040 (purity of inactive test substance, not reported)
Dose levels	:	Original data not available. The concentration of the test substance was approximately 0,5% in this experiment, because the finale hair dye formulation was mixed with hydrogen peroxide containing developer.
GLP	:	QA statement included

Intact, clipped dorsal skin was exposed for 30 minutes to the ^{14}C labelled test substance in the hair dye formulation without occlusion. The content of 1,2,3,4-tetrahydro-6-nitroquinoxaline in the finale formulation after mixing with the hydrogen peroxide was approximately 0,5%. With a test area of 9 cm^2 the dose of test substance was approximately 0,11 mg/cm^2. Urine and faeces were taken as fractions for 0-24 hours, 24-48 and 48-72 hours. After 72 hours the animals were killed, and the radioactivity was determined in different layers of the treated skin, in special organs, in the carcass and in the excretions.

Results

After analysis 0.11 μg/cm^2 of 1,2,3,4-tetrahydro-6-nitroquinoxaline were percutaneously absorbed. 0.75 μg/cm^2 remained in the skin after tape stripping and is taken to be systemically available. The radioactivity was excreted mainly via urine (about 58%) and to a lesser extent via faeces (about 42%) with a total balance of 98%. Most of the radioactivity was eliminated within the first 24 hours after application (72%).

Ref. : 16

2.8. Mutagenicity/Genotoxicity

Bacterial gene mutation

The report includes the results of two experiments, one performed in March 1988, and the second during August 1990. This procedure is very unusual, and not conforming to any international

regulation. The two experiments were performed with two different samples of the hair dye B-104 (1st test: batch 2665/188; purity not stated; 2nd test: Batch 3933/58; purity=99.5%. Moreover, the test substance was tested as hydrochloride in the first test and as a free base in the second test.

The two tests are performed on *S. typhimurium* strains TA1535, TA100, TA1537, TA1538 and TA98 in the presence or absence of the metabolic activation system $S90^-$ mix obtained from liver of rats treated with Aroclor 1254 at a dose of 500 mg/kg.
Doses were between 8 and 500 µg/plate in the first test and between 4 and 2500 µg /plate in the second test. The solubility of the hair dyes was different in the two tests.

The hair dye was found to be mutagenic with and without S9-mix in strains TA1537, TA1538 and TA98 in both tests.

Ref. : 10

Mammalian cells gene mutation

The test is based on the induction of gene mutations at the HPRT locus in V79 (Chinese Hamster) cells *in vitro*. The methodology employed was that of the OECD Guideline no.476 and EC-B17 Guideline in the presence or absence of S-9 mix obtained from livers of Wistar rats treated with 500 mg/kg of Aroclor 1254.

The test compound of 95% purity (Ro1135, Batch 3359/28) hydrochloride was dissolved in DMSO, at the doses of between 10 and 125 µg /ml with or without S-9 mix. The positive controls were represented by EMS (1mg/ml) and by DMBA (15.4 µg /ml).
The induction indicated that the test compound was not mutagenic in this system.

Ref. : 11

Mammalian cell cytogenetic test

The test is based on the induction of chromosome aberration in V79 (Chinese Hamster) cells *in vitro*.
The methodology employed was that of the OECD Guideline no.473 and EC-B10 in the presence or absence of S-9 mix obtained from liver of Wistar rats treated with 500 mg/kg of Aroclor 12254.
The test compound of 98% purity (RO1135, Batch 3359/100) hydrochloride was dissolved in DMSO, at the doses 300 µg /ml (7h), 25; 100; 300 µg /ml (18h) and 300 µg /ml (28h) without S-9 mix, at the doses 100 µg /ml (7h), 10; 100; 200 µg /ml (18h) and 200 µg /ml (28h) with S-9 mix. Two parallel cultures were treated for each dose and condition of metabolic activation. The treatment lasted 4 hours. The positive controls were represented by EMS (without S-9 mix)) and DMBA (in the presence of S-9 mix).

The results indicated that the test compound was clastogenic on both methodological conditions at different doses and time of harvesting.

Ref. : 12

Unscheduled DNA synthesis in primary rat hepatocytes in vitro

The test is based on the induction of unscheduled DNA synthesis in rat hepatocytes treated *in vitro*. The methodology employed was that of the OECD Guideline no.482 and EC-B18. The test compound of 98% purity RO1135, Batch 3359(100), hydrochloride was dissolved in DMSO, at the doses between 5 and 500 μg /ml.
The hepatocytes, obtained freshly from Wistar male rats, were treated for 3 hours, in two independent experiments. The amount of UDS was determined by the application of Trypan Blue and by measuring the incorporation of ^{3}HTdR. As positive control was used 2-AAS at a dose of 22.32 μg /ml.

The results indicated in general that the test compound, besides some marginal but statistically significant induction of UDS at the dose of 50 μg /ml at the 5% level, did not induce genotoxic lesion detectable by this system. Only one time tested.

Ref. : 13

Micronucleus test in bone marrow cells of the mouse

The test is based on the induction of Micronuclei in bone marrow cells of the mice treated *in vivo*. The methodology was that of the OECD Guideline no.470 and EC-B12.
The test compound of 98% purity 5291, Batch no.3359/100, hydrochloride, was dissolved in water and administered at the dose of 250 mg/kg bw by oral intubation (5 males and 5 females/group).
The animals were sacrificed 24, 48 and 72 hours after treatment. Cyclophosphamide at 50 mg/kg bw was employed as positive control.
The results indicated that there was no increase in the frequency of micronuclei in all the groups treated with the test compound, compared to the negative controls, whereas Cyclophosphamide was known to induce micronuclei.
The ratio polychromatic/normochromatic erythrocytes was not decreased, due to the treatment, thus indicating that the bone marrow cells might not have been exposed to the test compound.
The dose of 250 mg/kg was selected on the basis of a pilot study, when the animals were dosed with 1000, 500, 250 and 50 mg/kg (3 males and 3 females/group). The animals, treated with 250 mg/kg were lethargic and showed pilo-erection up to 2 days after treatment; they were alive after 3 days; the animals treated with 1000 and 500 mg/kg died during the first day after treatment (Swiss mice, CD-1).
In rats a medium acute lethal dose (LD_{50}) of 1,058 mg/kg bw, has been reported in Ref. 1.

Ref. : 14

Conclusions :

The test compound, tetrahydro-6-nitroquinoxaline (B104) has been found mutagenic in :

* Bacterial gene mutation test (as a free base and hydrochloride cells)
* *In vitro* mammalian cell chromosome test.

Moreover, the test compound has been found marginally positive (at 1-2 doses) in the UDS *in vitro* rat hepatocytes, but classified as a non genotoxic.

The test compound has been found non mutagenic in the *in vitro* mammalian cell gene mutation test.
The test compound has been found non mutagenic in the induction of micronuclei in bone marrow cells of mice treated *in vivo*, but there was no indication of exposure to the test compound of the mice bone marrow cells.
Due to the considerable mutagenic activity observed in strains TA1537, TA1538 and TA98 of *S. typhimurium* in the presence and absence of S9-mix metabolic activation system (100 fold increase in the number of revertants) and to the considerable cytogenetic effect observed in all doses, all conditions and all time-intervals in mammalian cells treated *in vitro*, similar to the quantitative results obtained with the two positive controls, the compound has to be considered an *in vitro* mutagen.

2.9. Carcinogenicity

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2.10. Special investigations

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2.11. Safety evaluation

2.11.1 Oxidation hair dye formulation

CALCULATION OF THE MARGIN OF SAFETY

(Tetrahydro-6-nitroquinoxaline)

(Oxidative)

Basing on an usage volume of 50 ml of hair dye formulation at a maximum concentration of 1 % tetrahydro-6-nitroquinoxaline in absence of developer mix, the maximum amount of ingredient applied would then be 500 mg.

Highest penetration	**PA (μg/cm^2)**	**=**	**0.9 μg/cm^2**
Typical body weight of human		**=**	**60 kg**
Exposed area (scalp)		**=**	**700 cm^2**
Systemic exposure	**PA x 700 cm^2**	**=**	**630 μg**
Systemic exposure dose (SED)	**PA x 700/ 60 x 1000**	**=**	**10.5 μg/kg bw**
No observed adverse effect level (mg/kg) (species, route of application)	**NOAEL**	**=**	**5 mg/kg bw**
Margin of Safety	**NOAEL / SED**	**=**	**476**

2.11.2 Non-oxidation hair dye formulation

CALCULATION OF THE MARGIN OF SAFETY

Tetrahydro-6-nitroquinoxaline

(Non-oxidative)

Basing on an usage volume of 35 ml of hair dye formulation at a maximum concentration of 1 % tetrahydro-6-nitroquinoxaline in absence of developer mix, the maximum amount of ingredient applied would then be 350 mg.

Highest penetration	**PA ($\mu g/cm^2$)**	**=**	**2.5 $\mu g/cm^2$**
Typical body weight of human		**=**	**60 kg**
Exposed area (scalp)		**=**	**700 cm^2**
Systemic exposure	**PA x 700 cm^2**	**=**	**1750 μg**
Systemic exposure dose (SED)	**PA x 700/ 60 x 1000**	**=**	**29 $\mu g/kg$ bw**
No observed adverse effect level (mg/kg) (species, route of application)	**NOAEL**	**=**	**5 mg/kg bw**
Margin of Safety	**NOAEL / SED**	**=**	**172**

2.12. Conclusions

* 1,2,3,4-tetrahydro-6-nitroquinoxaline hydrochloride is neither corrosive nor irritant to rabbit skin. However, the undiluted test substance (hydrochloride) was strongly irritant to the eye of the rabbits, the undiluted free base was less irritant.
After repeated topical applications of 1,2,3,4-tetrahydro-6-nitroquinoxaline hydrochloride, no adverse effects were noted.

* The substance was determined to be a potent allergen in the Guinea Pig Maximization Test.

* Strong positive reactions were observed *in vitro* with Ames tests (with the free base as well as with hydrochloride) and with chromosome aberration assay. Negative results have been obtained *in vitro* with the HPRT-*locus* assay and in the *in vitro* induction of unscheduled DNA-synthesis. The results of the *in vivo* micronucleus test performed with 1,2,3,4-tetrahydro-6-

nitroquinoxaline hydrochloride were also negative, with no indication of toxicity in the target cell.

* Based on the results obtained from the sub-chronic toxicity studies conducted by oral route in the rat, a NOEL of 5 mg/kg/day was obtained with the free base.
The reproductive study performed by the oral route in the rat revealed none teratogenic, embryolethal or embryotoxic effects with 1,2,3,4-tetrahydro-6-nitroquinoxaline.

* An *in vivo* assay has been conducted on Rat skin showing an absorption rate of tetrahydro-6-nitroquinoxaline amounted 0.9 µg after 30 minutes contact to the skin with a basic cream formulation (without developer mix) and amounted 2.5 µg after 30 minutes contact to the skin with a realistic formulation (with developer mix).

2.13. Opinion

The SCCNFP is of the opinion that the information submitted is insufficient to allow an adequate risk assessment to be carried out.

Before any further consideration, additional data would be required on in vivo mutagenicity/genotoxicity.

2.14. References

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