

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

CONCERNING

PEG-3,2',2'-DI-P-PHENYLENEDIAMINE

COLIPA n° A146

adopted by the SCCNFP during the 25th plenary meeting
of 20 October 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 PEG-3,2',2'-di-p-Phenylenediamine 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

PEG-3-2',2'-di-*p*-phenylenediamine (INCI)

2.1.2. Chemical names

Chemical names : 1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane x 4 HCl
 CAS name : 1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane tetrahydrochloride
 Synonyms : 1,10-bis-(2,5-diaminophenyl)-triethyleneglycol tetrahydrochloride

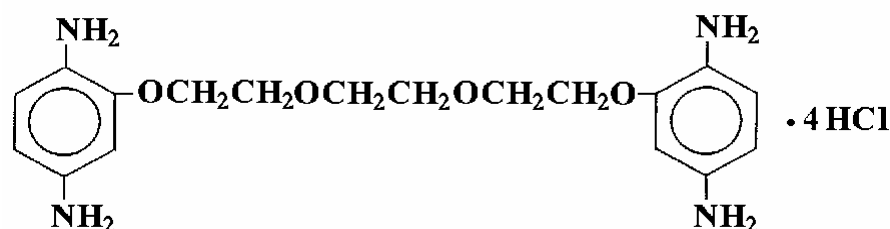
2.1.3. Trade names and abbreviations

Trade name : Ro 1227
 COLIPA n° : A 146

2.1.4. CAS No. / EINECS No.

CAS no : 144644-13-3
 EINECS : /

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula : $C_{18}H_{26}N_4O_4 \times 4 HCl$
 Mol weight : 362.42 (free base)
 508.28 (tetrahydrochloride)

2.1.7. Purity, composition, and substance codes

Purity

Titre as determined by HPLC : > 99% (peak area)
 Water content : /
 Heavy metals : /

Evaluation and opinion on : PEG-3,2',2'-di-*p*-Phenylenediamine

Potential impurities	:	/
Reagents and intermediate reaction products	:	/
Solvent residues	:	/

2.1.8. Physical properties

Appearance	:	Yellowish/greyish powder, odourless
Melting point	:	233-234 °C
Boiling point	:	/
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{ow}	:	/

2.1.9. Solubility

Soluble in water

2.1.10 Stability

Four hours in an aqueous solution and in DMSO

General comments on analytical and physico-chemical characterisation

- * Several batches of PEG-3-2',2'-di-*p*-phenylenediamine tetrahydrochloride have been used for various tests, purity > 98%. The purity is checked by HPLC with UV detection at 265 nm, while the λ_{max} according to the submitted UV spectrum is 204 nm/237 nm/286 nm. HPLC peak detection is performed at 265 nm, which is a trough between 237 nm and 286 nm. The peak area count has been considered as the measure of purity/impurity. Absolute concentration of the dye in the test material is not reported.
- * No information on impurities is provided. An impurity (0.7%, peak area) in the HPLC chromatogram is seen, but the impurity remains unidentified.
- * There is no mention of solvent residues, if any.
- * Log P_{OW} of the test material is not reported.
- * No quantitative data is given for solubility.
- * Limited data is provided on stability; suggestion of chemical sensitivity to light and moisture; at least on one occasion the stability in solvent reported as unknown.

2.2. Function and uses

PEG-3-2',2'-di-*p*-phenylenediamine tetrahydrochloride is used as oxidation dye in hair dye formulations up to a final concentration of 2.5 % in combination with a developer-mix and up to 2.5 % in the absence of this reactive mix.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Guideline	:	OECD 401 (1987)
Species/strain	:	Wistar rat
Group size	:	5 males + 5 females
Test substance	:	1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane x 4 HCl (Ro 1227) in distilled water
Batch no	:	3933/155
Purity	:	> 99%
Dose	:	500, 1000, and 2000 mg/kg bw by gavage
Observ. Period	:	14 days
GLP	:	in compliance

5 male (body weight 198-235 g) and 5 female (body weight 152-182 g) Wistar rats per dose group were treated with 500, 1000, and 2000 mg/kg bw of the test substance by gavage.

Results

At the dose 2000 mg/kg bw all animals died while one female died following treatment with 1000 mg/kg bw and none at 500 mg/kg bw. The animals of the dose group 1000 mg/kg bw showed lower than expected body weight gain or even weight loss. In the 500 mg/kg bw dose group the body weight gain was similar to normal untreated animals. Clinical signs of toxicity were found in the 1000 mg/kg bw group. On the day of dosing urine was found red/brown discoloured in all dose groups. The LD₅₀ of 1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane x 4 HCl administered to rats by the oral route is between 1000 and 2000 mg/kg bw.

Ref. : 1

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

No data

2.3.5. Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Sub-chronic oral toxicity

Guideline	:	OECD 408 (1981)
Species/strain	:	Sprague-Dawley Ico:OFA.SD(IOPS Caw)
Group size	:	10 males + 10 females
Test substance	:	1,10-Bis-(2,5-diaminophenyl-1,4,7,10-tetraoxadecane x 4 HCl (Ro 1227) in water pro injectione
Batch number	:	4490/26
Purity	:	> 98 %
Dose levels	:	0, 10, 25, 60 mg/kg bw by gavage, once daily
Exposure period	:	13 weeks
GLP	:	in compliance

20 rats (10 per sex) were used per dose and control group and an additional group of 10 rats (5 per sex) was included (high dose and control group) for 4 weeks without treatment (recovery group). The test substance was administered by gavage, once daily for 13 weeks. The control group received the vehicle (water for injection) only. All animals were observed twice daily for clinical signs and mortality, body weights were recorded individually in weekly intervals. At the end of the study, blood and urine samples were taken from all animals for haematological and clinical chemistry investigations as well as for urinalysis. Ophthalmoscopic examination was performed on all animals at pretest and at the end of the study on all control and high dose animals. All animals were sacrificed at the end of the study, organ weights were recorded, macroscopy and histopathology were performed.

Results

No animal died during the study. Animals of the dose groups 25 and 60 mg/kg bw had coloured urine. Body weight gain, food and water consumption did not show any substance related change. No abnormal findings were noted at ophthalmoscopy.

Only minor changes were found in some haematological and clinical biochemistry parameters. Urine of the high dose animals was darkly coloured and contained epithelial cells, and in males more blood. These changes were reversible as seen in the recovery group. In the high dose group all animals and some animals of the 25 mg/kg bw group showed mainly intracellular brownish pigmentation of the kidney tubules. Furthermore, pigmentation was observed in the thyroid epithelium and in the stroma of the villi of the anterior part of the duodenum. No pigmentation was seen in the target organs of the group 10 mg/kg bw. Only in the high dose group an inflammatory change of the forestomach was observed in males.

The NOAEL in this study is 10 mg/kg bw.

Ref.: 13

2.3.8. Sub-chronic dermal toxicity

No data

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

No data

2.4. Irritation and corrosivity**2.4.1. Irritation (skin)****Acute Dermal irritation**

Guideline	:	OECD 404 (1981)
Species/strain	:	New Zealand White rabbits
Group size	:	3 females
Test substance	:	RO 1227
Batch number	:	3933/155
Purity	:	>99%
Dose	:	0.5g of moistened substance
GLP	:	In compliance

Approximately 24 hours prior to treatment, the dorsal fur was shaved, to expose an area of about 10cm².

An aliquot of 0.5g of the moistened test substance was applied, semi-occlusively for 4 hours beneath a 2 x 3 cm patch, to the intact shaved back of each animal. Animals were examined for signs of erythema, and oedema formation at approximately 1 hour, 24, 48 and 72 hours after removal of the patches. Each animal served as its own control. There was no staining of the skin by the test material.

Results

Very slight erythema was observed in all 3 animals and very slight oedema in 1 animal. All signs had gone by the 24 hour observation. A primary irritation index of 0 was determined indicating that the substance was classified as non-irritant to rabbit skin.

Ref. : 2

Dermal irritation in rabbits after repeated application

Guideline	:	/
Species/strain	:	New Zealand White rabbits
Group size	:	6 females
Test substance	:	RO 1227
Batch number	:	3933/155
Purity	:	>99%
Dose	:	0.05ml of 5% aqueous solution (pH adjusted to 8-10 with NaOH), spread over 6cm ² .
GLP	:	In compliance

The study was performed according to the method of Marzulli and Maibach (1975).

Approximately 24 hours prior to treatment, the dorsal fur was shaved, to expose an area of about 10cm².

Using a glass rod, a volume of 0.05ml of a 5% aqueous solution of the test material was applied over an area of 6cm² of each animal. The treatment was repeated on 5 consecutive days each week for 3 weeks.

The animals were examined daily for signs of erythema and oedema. There was black coloration of the skin at the site of application of the test material at all observations.

Results

During and after the application, 3 animals showed a very slight erythema. The substance was considered as being non-irritant to rabbit skin under the test conditions.

Ref. : 5

2.4.2. Irritation (mucous membranes)

Guideline	:	OECD 405 (1981)
Species/strain	:	albino rabbits, New Zealand
Group size	:	3 males
Test substance	:	RO 1227
Batch number	:	3933/155
Purity	:	>99%
Dose	:	0.1ml 5% w/w aqueous dilution (pH adjusted to 9.6 with NaOH)
GLP	:	In compliance

A volume of 0.1ml of a 5% aqueous dilution of the test substance was instilled into the conjunctival sac of the left eyes of the test animals. The right eyes served as controls. The test substance remained in contact with the eyes until rinsing with a 2% fluorescein solution 24 hours after instillation. The eyes were examined 1 hour, 24, 48 and 72 hours after instillation of the test material.

Results

The test material did not cause any observable effect on the corneas or irises at any time. Slight conjunctival redness was observed 1 hour after instillation in all 3 animals; this had resolved by 24 hours. There was some black staining around the eyes.

Under the conditions of the test, the test material was considered to be minimally irritating to the eye in this animal model.

Ref. : 4

In vitro Irritation Potential – HET-CAM

Guideline	:	/
Species/strain	:	fertilised fresh chicken eggs
Group size	:	6
Test substance	:	RO 1227
Batch number	:	3933/155
Purity	:	>99%
Dose	:	25% in physiological saline.

GLP : In compliance

A 25% dilution of the test substance was exposed to the CAM of each prepared egg. The substance remained in contact with the CAM for 30 seconds and then rinsed off with physiological saline.

Results

The 25% dilution of the test substance caused slight irritant effects to the CAM of fertilised chicken eggs. Based on these results, it was considered that a 25% aqueous dilution of the test substance would be only slightly irritating to mucous membranes.

Ref. : 3

2.5. Sensitisation

Guinea pig maximisation test (Magnusson and Kligman)

Guideline	:	OECD 406	
Species/strain	:	Pirbright White guinea pigs	
Group size	:	20 females (experimental), 10 females (control), 5 females (range-finder)	
Test substance	:	RO 1227	
Batch number	:	3933/155	
Purity	:	>99%	
Dose	:	Intradermal induction	: A 2.5% aqueous solution with and without Freund's Complete Adjuvant.
		Topical induction	: A 50% dilution of test material under occlusion for 48 hours. Controls received vehicle only.
		Challenge	: 14 days later by exposing 5%, 2%, and 1% aqueous dilution of the test substance (24 hours, occlusion) to the animal flanks.

GLP : In compliance

Animals were examined 24 and 48 hours after removal of the patches for signs of erythema and oedema.

Results

The test substance caused irritant effects in the two induction periods.

The 5% dilution was found to be irritant based on the effects seen in the controls (8 animals showed a response). In the experimental group, all 20 animals reacted to 5%, 2% and 1%.

Based on the results of the above, the test substance was classified as an extreme sensitiser under the conditions of the test in the guinea pig.

Ref. : 6

Buehler test

Guideline	:	OECD 406 (1981)
Species/strain	:	Guinea pigs, albino Dunkin-Hartley
Group size	:	40 animals. 10 of either sex, in the experimental and control groups.
Test substance	:	RO 1227
Batch number	:	3933/155

Evaluation and opinion on : PEG-3,2',2'-di-p-Phenylenediamine

Purity	:	>99%	
Dose	:	Topical induction	: 0.5% aqueous solution (61% w/w)
		Challenge	: 0.5% aqueous solution (61% w/w)
GLP	:	In compliance	

The topical induction was performed on one shaved trunk side on days 1, 8 and 15 by applying 0.5ml of an aqueous solution (61% w/w) under occlusive conditions for 6 hours. Control animals received equal amounts of purified water.

The challenge was carried out 14 days later by applying 0.5% of an aqueous solution (61% w/w) occlusively for 6 hours to the untreated flanks.

Animals were examined 24 and 48 hours after removal of the patches for signs of erythema and oedema.

Results

There were traces of blackish coloration at the sites of application of the test material. There were no macroscopic changes. Under the conditions of the test, the test substance did not cause any evidence of sensitisation in this animal model.

Comment

The test concentrations were very low and the study is considered inadequate.

Ref. : 7

2.6. Teratogenicity

Guideline	:	EEC council recommendations for medicinal products (1983)
Species/strain	:	Sprague-Dawley rat
Group size	:	25 females mated per dose group
Test substance	:	Ro 1227 in water
Batch number	:	4490/26
Purity	:	> 98%
Dose levels	:	0, 10, 45, and 200 mg/kg bw by gavage
Treatm. period	:	Day 6 - 15 of gestation
GLP	:	in compliance

The test substance was administered, once daily by gavage, from day 6 to 15 of gestation, to groups of 25 pregnant rats at the doses 0, 10, 45, and 200 mg/kg bw, respectively, based on the results of a dose range-finding study. The control group received the vehicle (water) only. The animals were observed at least once daily for mortality and clinical signs. Individual body weights were reported for days 0, 6, 16, and 20 of gestation. Food consumption was measured for the day-intervals 0-6, 6-16, and 16-20. All mated females were sacrificed at day 20 of gestation.

Following sacrifice, macroscopic examination of the maternal organs was carried out. The uterus was removed and weighed, the number of corpora lutea, early and late resorptions, total implantations and viable foetuses were recorded. All foetuses were individually weighed, examined for external abnormalities and the sex of the foetuses was determined. Half of the foetuses were examined for skeletal defects and variations of the ossification process by Alizarin Red staining and one half was evaluated for visceral alterations.

Results

Only in the high dose group (200 mg/kg bw) maternal food consumption and body weight gain were reduced. The majority of the females in this dose group had dark kidneys and/or black points on the kidneys.

No substance-related changes of reproduction data (number of implantations, resorptions and foetuses, foetal weight and external abnormalities) were noted. No substance-related changes in the incidence of visceral and skeletal abnormalities were found, but the reporting of skeletal abnormalities does not allow an evaluation in detail.

The NOAEL of embryo/foetotoxicity was 200 mg/kg bw, the NOAEL of maternal toxicity was 45 mg/kg bw in this study.

Ref.: 14

2.7. Toxicokinetics (including Percutaneous Absorption)

Guideline	:	/
Tissue	:	Rat clipped dorsal skin (female rats Sprague Dawley strain)
Method	:	<i>in vivo</i> measurement of the absorption from the urinary and faecal excretion, and from the skin residue, carcass and excreta analysis after sacrifice of the animal
Test substance	:	1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane (x4 HCl) at the concentration of 4 % in a commercial type formulation (pH 9.5 – 10)
Batch no	:	4490/47
Purity	:	/
Dose	:	200 mg over 9 cm ² , i.e. 0.46 mg of 1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane (x4 HCl) /cm ²
Replicate	:	6 rats per excipient
Analyt. method	:	liquid scintillation, ring ¹⁴ C-1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane (x4 HCl) (radiochemical purity from the supplier : 95.3 %)
Detection limit	:	0.002 % to 0.00001 %/g according to the structure analysed
Stability ingredient	:	no information concerning the stability of 1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane (x4 HCl) in the formulation according to time
GLP	:	in compliance

The skin penetration of 1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane (x4 HCl) was evaluated *in vivo* in rats. The test substance was prepared at a concentration of 4 % in a “commercial type” formulation. Immediately before the application the hair dye was mixed (1:1) with water “study excluding a developer” or with a developer dispersion, containing 6 % hydrogen peroxide “study including a developer”. After dilution, 4.16 mg of the test substance, i.e. 0.46 mg/cm² (200 mg of the formulation), were applied to 9 cm² of the dorsal skin for 30 minutes without occlusion. The excess from the skin surface was scraped off using a spatula, then rinsed off with water and finally dried with absorbent cellulose. The treated area was covered by a non occlusive dressing to prevent licking of the skin by the animals (individually housed in metabolic cages) during the 72 hours of the excretion kinetic study. Urine and faeces were collected by periods of 24 hours. 72 hours after the animals were sacrificed, the skin of the application site was collected, hair, stratum corneum and dermis were separated for individual analysis. Specific organs and the carcass were analysed separately. ¹⁴C-1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane (x4 HCl) and related compounds were globally assayed by liquid scintillation.

Results

For the formula without a developer, the quantity of test substance penetrating through the skin, evaluated from the urinary and faecal excretions and from the amount recovered in the whole carcass and the organs was $0.28 \% \pm 0.21 \%$ of the applied dose. This is corresponding to $1.3 \mu\text{g}$ equivalent of the test substance absorbed/ cm^2 . If one considers the residual amount recovered in the dermis ($0.14 \% \pm 0.068 \%$) of the application area as absorbed, the total absorption corresponds to 0.42% of the applied dose (i.e. $2 \mu\text{g}/\text{cm}^2$). The majority of the radioactivity was recovered in the excretions. Elimination was fast, 76% of the total was excreted within 24 hours. The mass balance of the study was $96.3 \% \pm 2.4 \%$ of the applied dose.

For the formula with the developer, the total absorption was lower, $0.067 \% \pm 0.051 \%$ of the applied dose (i.e. $0.31 \mu\text{g}$ equivalent / cm^2). If the residual amount still present in the dermis is considered absorbed ($0.042 \% \pm 0.044 \%$) the total amount absorbed is 0.109% (i.e. $0.50 \mu\text{g}$ equivalent / cm^2). The excreted amount via faeces was larger (63.9% of the total eliminated amount) than via urine (36.1% of the total eliminated amount). The mass balance of the study was $93.2 \% \pm 2.9 \%$ of the applied dose.

The test was performed *in vivo* in the rat and is technically corresponding to the current OECD guidelines.

Ref. : 11, 12

2.8. Mutagenicity/Genotoxicity

2.8.1. Mutagenicity/Genotoxicity *in vitro*

Bacterial Reverse Mutation Test

Guideline	:	OECD 471
Species/strain	:	<i>Salmonella typhimurium</i> , TA98, TA100, TA1535, TA1537, TA 1538
Replicates	:	Triplicate plates, 2 independent tests
Test substance	:	1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane x 4 HCl
	:	Ro 1227
Batch no	:	1 st test : 3933/115 (no information given) 2 nd test : 3933/152, purity : 98.6 % (HPLC)
Stability	:	in solvent : air sensitive at pH > 7
Concentrations	:	Experiment # 1 and # 2 <i>Salmonella typhimurium</i> and <i>E. coli</i> With or without metabolic activation Test #1 : 8, 40, 200, 1000, 5000 $\mu\text{g}/\text{plate}$ 1.1, 3.3, 10, 30, 90 $\mu\text{g}/\text{plate}$
GLP	:	In compliance

A 146 (1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane x 4 HCl) has been investigated for gene mutation in *Salmonella typhimurium* using the direct plate incorporation method both with or without S9 mix. Purchased S9 mix was obtained from rats injected i.p. with AroclorTM 1254 and was characterised for protein content.

Results

Toxicity : toxicity was noted at $1000 \mu\text{g}/\text{plate}$ and higher.

Revertant number

Test # 1

In the absence of activation, no dose related relevant increase in revertant numbers was observed. In the presence of activation, an increase in revertant numbers was observed in the TA 1538.

Test # 2

In the absence of activation, no dose related relevant increase in revertant numbers was observed. In the presence of activation, an increase in revertant numbers was observed in the TA 1538 and TA 98 tester strains.

Positive controls showed the expected responses

Conclusions

Based on the reversion rate, and under the conditions of the assays performed, it is concluded that the test substance shows evidence of mutagenic activity in some tester strains in the presence of S9 mix. It should be noted that discrepancies exist between the raw data and the summary.

Ref. : 8

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

OECD guideline :	OECD 476 (1984)
Species/strain :	V79 cell line / HPRT Locus
Replicates :	2 independent tests with and without metabolic activation
Test substance :	(1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane x 4 HCl)
	Ro 1227
Batch no :	3933/173
Purity :	> 98 % (method not indicated by the sponsor)
Stability :	pure : months
	in water and DMSO : 4 hours
Concentrations :	Experiment # 1 and # 2
	Without metabolic activation
	0.3, 1, 2, 3, 6 and 10 µg/ml
	With metabolic activation
	10, 30, 100, 200, 400, 600 µg/ ml
	Experiment # 3
	Without metabolic activation
	1, 2, 3 and 4 µg/ml
Treatment time :	4 hours
GLP :	In compliance

A 146 (1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane x 4 HCl) has been investigated for gene mutation in the HPRT locus of V79 Chinese hamster cell lines, both with or without S9 mix.

S9 mix was obtained from male Wistar rats injected i.p. with AroclorTM 1254. Negative and positive controls were in accordance with the OECD guideline.

Results

Solubility : not described

Osmolarity : Osmolarity measurement of post treatment medium was not performed.

Plating efficiency

Test # 1 & 2 with or without S9 mix

Relative Plating efficiency was reduced at acceptable levels for the top doses (Exp # 1 - without S9 : 25 %, with S9 : 18 % and Exp # 2 - without S9 : 24 %, with S9 : 18.9 %).

Mutant frequencies

Test #1 In the absence of activation

A significant increase in mutant colony numbers was observed for the dose of 3.0 µg/ml.

Test #1 in the presence of activation

No significant increase in mutant colony numbers was observed for any doses.

Test #2 in the absence or presence of activation

No significant increase in mutant colony numbers was observed for any doses.

Test #3 in the absence of activation

No significant increase in mutant colony numbers was observed for any doses.

Conclusions

Based on the fact that the increase observed in one experiment at one dose has not been repeated, no biologically relevant significant increase in mutant colony numbers was observed over the concurrent solvent controls after treatment with (1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane x 4 HCl) in either test in the presence or absence of activation.

Therefore, the test substance does not demonstrate mutagenic potential on the HPRT locus in V79 cells.

Ref. : 9

2.8.2 Mutagenicity/Genotoxicity *in vivo*

Mammalian Erythrocyte Micronucleus Test

Guideline	:	OECD 474 (1981)
Species	:	OF1 mice
Group sizes	:	5 males and 5 females
Test substance	:	(1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane x 4 HCl)
	:	Ro 1227
Batch no	:	3933/167
Purity	:	Considered 100 % for the study
Dose levels	:	The test substance was administered by intragastric gavage. 1 single oral dose of 250 mg/kg bw for the 24, 48 and 72 h sacrifice time
Justification	:	Human possible route of exposure.
GLP	:	In compliance

A 146 (1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane x 4 HCl) has been investigated for induction of micronuclei in the bone marrow cells of male or female mice. A preliminary range finding study in which no observable clinical toxic effects were seen but mortality observed at 500 mg/kg and above.

The substance was administered by a single intragastric gavage and the groups of animals sacrificed 24, 48 or 72 hours after administration. Negative and positive controls were in accordance with the OECD guideline.

Number of cells scored : a total of at least 1000 erythrocytes were examined from each animal and the incidence of micronucleated erythrocytes determined. The ratio of polychromatic erythrocytes to normochromatic erythrocytes were calculated after the microscopic observations of 100 cells.

Results

In the main study, no death occurred and no clinical signs of toxicity were noted but the urine was coloured on day 2.

PCE/ NCE : no significant change in the ratio was observed after treatment as compared to controls for all sacrifice times.

Micronucleated PCE 24, 48 or 72 hours sampling time : no statistically significant or biologically relevant increase in the incidence of micronucleated polychromatic erythrocytes over the concurrent vehicle control values were observed for any sacrifice times.

Conclusions

Under the conditions of the test it can be concluded that A 146 (1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane x 4 HCl), at a dose at which no signs of clinical toxicity were recorded, does not induce statistically significant increase in the frequency of micronucleated PCE at any sacrifice times interval. Toxicokinetic data indicate that the substance reaches the bone marrow. Therefore, A 146 (1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane x 4 HCl) is not clastogenic and/or aneugenic in this mouse bone marrow micronucleus test.

Ref. : 10

2.9. Carcinogenicity

No data

2.10. Special investigations

No data

2.11. Safety evaluation

Not applicable

2.12. Conclusions

The absolute content of the test substance in all batches and the impurities in the test material are not described. Log P_{ow} of the test material is not reported, and the information provided on solubility and stability of the test substance is insufficient.

The oral LD_{50} of A 146 in rats is between 1000 and 2000 mg/kg bw. The target organ in the 13 weeks oral toxicity study in rats was mainly the kidney showing brownish pigmentation in the tubuli. The NOAEL was 10 mg/kg bw. The NOAEL of embryo/foetotoxicity in a rat study was 200 mg/kg bw.

The substance was classified non-irritant to the skin and slightly irritant to the eye. It was classified as an extreme sensitiser under the conditions of the test in the guinea pig.

The percutaneous absorption was performed *in vivo* in the rat.

For the formula *without* a developer, the absorption was set at 1.3 µg/cm². If one considers the residual amount recovered in the dermis as absorbed, the total amount absorbed is 2 µg/cm².

For the formula *with* the developer, the total absorption was set at 0.31 µg/cm². If the residual amount still present in the dermis is considered absorbed, the total amount absorbed is 0.50 µg/cm².

A 146 (1,10-bis-(2,5-diaminophenyl)-1,4,7,10-tetraoxadecane x 4 HCl) has been tested in prokaryotic and mammalian cells for gene mutation and in one *in vivo* bone marrow micronucleus test. The *in vitro* test for gene mutation in prokaryotes with the test substance has been found positive in the presence of metabolic activation system. The *in vitro* test for gene mutation in mammalian cells showed that the test agent is non mutagenic under both activation conditions. The *in vivo* micronucleus test in mice gave negative results.

The data provided are insufficient for a final evaluation.

2.13. References

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3. Opinion of the SCCNFP

The SCCNFP is of the opinion that the information submitted is inadequate to assess the safe use of the substance.

Before any further consideration, the following information is required :

- * Complete characterisation of impurities in the test material; Log P_{ow}, quantitative data on solubility in relevant solvents including the receptor fluid; information on stability of the test material in the test solutions and hair dye formulations.
- * data on the genotoxicity/mutagenicity following the relevant SCCNFP-opinions and in accordance with the Notes of Guidance.

4. Other considerations

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5. Minority opinions

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