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

CONCERNING

DISPERSE VIOLET 1 IMPURIFIED WITH DISPERSE RED 15

Colipa n° C64 & C61

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 Disperse Violet 1 impurified with Disperse Red 15 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

Disperse Violet 1 impurified with Disperse Red 15 (INCI names)

2.1.2. Synonyms

Disperse Violet 1

1,4-Diamino-anthraquinone
1,4-Diamino-9,10-anthracenedione
CI 61 100

Disperse Red 15

1-Hydroxy-4-amino-anthraquinone
1-Hydroxy-4-amino-9,10-anthracenedione
CI 60 710

2.1.3. Trade names and abbreviations

Disperse Violet 1

e.g. : Mühlacetviolett 2R, Celliton-Rotviolett RN, Cellitoneechtrotviolett RN, Lidoechtrotviolett

Disperse Red 15

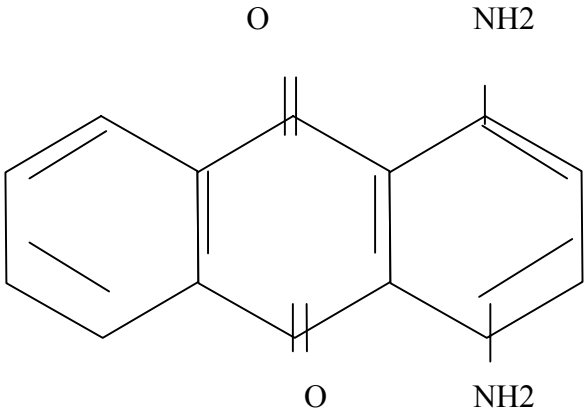
e.g. : Acetquinonlichtgroseille RL, Acetoquinoneechtlichtgroseille, Cellitonechtrosa

2.1.4. CAS no.

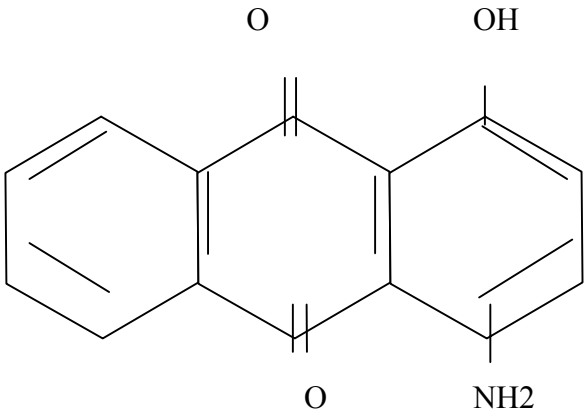
128-95-0 (Disperse Violet 1)
116-85-8 (Disperse Red 15)

2.1.5. Structural formula

C 64



C 61



2.1.6. Empirical formula

Disperse Violet 1

Emp. Formula : $C_{14}H_{10}N_2O_2$
Mol weight : 238.25

Disperse Red 15

Emp. Formula : $C_{14}H_9NO_3$
Mol weight : 239.24

2.1.7. Purity, composition and substance codes

Disperse Violet 1 is a hair dye compound which is impurified with Disperse Red 15.

Disperse Violet 1 : 95.6 – 97.3 %
Disperse Red 15 : 2.7 – 4.4 %

2.1.8. Physical properties

Disperse Violet 1

Subst. Code :
Appearance : dark violet crystals, black powder
Melting point : 268 °C
Boiling point : /
Density : /
Rel. vap. dens. : /
Vapour Press. : /
Log P_{ow} : /

Disperse Red 15 : no data available

2.1.9. Solubility

Disperse Violet 1 : soluble in alcohol, benzene, pyrimidine, acetone and lineseed oil; forms suspension with water at 25°C.

Disperse Red 15 : no data available

2.2. Function and uses

Disperse Violet 1 impurified with Disperse Red 15 is used as a non-oxidising colouring agent for hair dyeing at a maximum concentration of 2.0 % in the finished cosmetic product.

TOXICOLOGICAL CHARACTERISATION

2.3. Toxicity

2.3.1. Acute oral toxicity

Acute oral toxicity studies of 1,4-Diamino-anthraquinone were performed in the Rat and in the Mouse. The calculated oral median lethal doses were :

- 3.50 g/kg body weight for female rats, 3.30 g/kg body weight for male rats and 820 mg/kg bw for mice

Ref. : 1

- above 5 g/kg bw in the rats

Ref. : 2

- 1800 mg/kg bw in the rats

Ref. : 3

- between 1250 and 5000 mg/kg bw for males rats and between 625 and 1250 mg/kg bw for female rats.

Ref. : 4a

Acute oral toxicity studies of 1-Hydroxy–4-amino-anthraquinone were performed in the Rat and in the Mouse. The calculated oral median lethal doses were :

- 6.65 g/kg bw for female rats, 3.55 g/kg bw for female mice.

Ref. : 34

2.3.2. Acute intraperitoneal toxicity

An acute intraperitoneal toxicity study of 1,4-Diamino-anthraquinone was conducted in NMRI mice.
Median lethal dose for 1,4-Diamino-anthraquinone was 640 mg/kg bw.

Ref. : 3

2.3.3. Acute inhalation toxicity

An acute inhalation toxicity study of 1,4-Diamino-anthraquinone was conducted in the Rat**.**
No mortality occurred, no adverse effects were recorded.

Ref. : 3

2.3.4. Repeated dose dermal toxicity

1,4-Diamino-anthraquinone, at a 5 % concentration in a hair dye formulation, was applied to the skin of 5 hairless mice once daily for 14 consecutive days.

Under the experimental conditions adopted, no adverse effects were observed with the tested preparation.

Ref. : 2

2.3.5. Sub-chronic oral toxicity

A 13-week oral toxicity study was conducted in 40 Wistar rats (2 groups, 10 animals per sex) with 1,4-Diamino-anthraquinone at a concentration of 500 ppm in the diet. The animals were examined for general state of health, body weight, blood investigations (haemoglobin content, red and white blood cell counts, calcium, blood sugar, urea), urine investigations (pH-value, specific gravity, protein, sugar, ketone bodies, blood), liver function (SAP, SGPT, SGOT), organ weights, histopathological examination.

There were no clinical signs considered related to the test article. No adverse effect was recorded. Body and organ weight were unaffected by the treatment. Blood chemistry, urinalysis parameters and liver function did not reveal pathological changes.

Ref. : 2

A 13-week oral toxicity study was conducted in 120 Sprague-Dawley rats (4 groups of 15 rats per sex) with a dye formulation containing 16 % 1,4-Diamino-anthraquinone and 2.5 % 1-Hydroxy-4-amino-anthraquinone administered into the diet at concentrations of 0.01 %, 0.0316 % and 0.1 % corresponding respectively to 0.00025 %, 0.00079 % and 0.0025 % of the test compound. The average intake of the test substances C64 and C61 over the whole period corresponded respectively to 1.14-1.28, 3.57-3.95 and 11.95–12.94 mg/kg/day and to 0.18-0.20, 0.56-0.62 and 1.87-2.02 mg/kg/day for the low, mid and high doses groups. No mortality was observed during the study. Animals of the mid and high dose groups showed blue-violet staining of fur. Body weight development and food consumption were unaffected by the treatment. Water consumption of high dose group female was significantly raised in week 2 and 8, this was attributed by investigators to be due to the generally higher body weight in this group. Differences between groups were noted concerning hematological (red cell and white cell count) and biochemical parameters (alkaline phosphatase), these data were not dose and time related, they were considered incidental by investigators. Liver weights were increased in male high and mid group and in female high group. Kidney weights were increased in male high group. These data were considered test article related. Concerning macroscopic and histopathological observations, the results were within the normal range of variation for this strain.

Based on these results, the NOAEL (No-Observed-Adverse-Effect-Level) was established to be 1.21 mg/kg (1.14 – 1.28) for 1,4-Diamino-anthraquinone and 0.19 mg/kg (0.18-0.20) for 1-Hydroxy-4-amino-anthraquinone.

Ref. : 15

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Guideline : OECD guideline 404 (1981)
Species/strain : Pirbright albino guinea pigs

Group size	:	5 female
Test substance	:	Disperse violet 1
Batch no	:	Not given (purity not stated in study report)
Dose	:	1 ml of a 1% aqueous formulation under closed patch
GLP	:	study not in compliance

The substance was suspended in water at a concentration of 1% and 1 ml was applied to 3 x 2 cm^2 area of intact skin of 5 female albino guinea pigs. Occlusive patches were applied and left in place for 4 hours. Reactions were evaluated after 24, 48, 72 and 144 hours.

Results
No cutaneous reactions were reported and the substance was classified as non-irritating to guinea pig skin at a concentration of 1% in water.

Ref . : 6

In one study 6 New Zealand white rabbits were used, and 500 mg disperse violet 1 was applied once as aqueous slurry to an area of 1 $inch^2$ of shaved intact skin. The material was left without occlusion for 24 hours, and the test reaction scored according to the Draize scale at 24 and 72 hours. All animals showed slight evidence of oedema and erythema at 24 hours, and none at 72 hours. The material was classified as a mild irritant (PII = 1,25) under these test conditions.

Ref. : 4c

Another experiment included 2 white Vienna rabbits. The test substance was prepared as a 80% aqueous formulation and rubbed onto the skin. Exposure time was 20 hours under semi-occlusive conditions, and readings were carried out after 24 hours and 8 days. There were no signs of irritation at both reading times.

Ref. : 3

5 hairless mice were exposed to a 5% formulation on the back once daily for 14 consecutive days. No reaction was seen. The composition of test material and type of bandage was not given. In the same reference is reported a human study including 7 volunteers who had closed patch tests on the upper arm with a 5% preparation once for 3 hours. Reading was performed after removal of the patch and 24 hours later. No reaction was seen.

Ref. : 2

2.4.2. Irritation (mucous membranes)

Guideline	:	Not given
Species/strain	:	New Zealand white rabbits
Group size	:	4 female
Test substance	:	Disperse violet 1(100 mg) neat substance
Batch no	:	Not given (purity not stated in study report)
Dose	:	100 mg neat substance
GLP	:	study not in compliance

The test substance was instilled in the conjunctival sack of the left eyes of the rabbits, and the right eyes were untreated. The eyes of 2 rabbits were rinsed off with 20 ml distilled water, 20 seconds after the instillation. One animal in the washed group died on day 3, and was replaced by another rabbit, which was treated the same way. All eyes were examined 1 hour and 1, 2, and 3 days post application. Scoring was done according to the Draize-method.

Results
The test substance caused redness, swelling and discharge in the eyes of all animals after 1 hour. By day 2 clearing was noted in 3 of 4 animals. All treated eyes were clear at day 3. Rinsed eyes were less irritated than those not rinsed. The test substance was classified as non-irritant in the rabbit eye irritation test.

Ref. : 4b

A summary of results from 2 other mucous membranes tests is included in the submission. The quality of the data cannot be established.

Rabbits were treated in the conjunctival sack with 2 drops of a 5% aqueous dye formulation and the animals were observed. No adverse reactions were noted. In another study 2 white Vienna rabbits were treated with 50 mg of the test substance into the conjunctival sack of their eyes and readings were done after 1 and 24 hours, and after 8 days. One hour after the application of the substance slight redness were observed, still visible after 24 hours, but not after 8 days.

Ref. : 2, 3

2.5. Sensitisation

Intra-cutaneous sensitisation test

Guideline	:	not given
Species/strain	:	Pirbright albino guinea pigs
Group size	:	15 animals in test group and 10 in control group
Test substance	:	Disperse violet 1
Batch no	:	Not given (purity not stated in study report)
Dose	:	1% aqueous solution
GLP	:	study not in compliance

The test animals were treated daily for 5 days with 3 intra-cutaneous injections of 0.1 ml of the test substance onto the flank. Four weeks later intra-cutaneous challenge was carried out on the opposite flank using a 1% aqueous solution diluted at 1-10, 1:100, 1:500, and 1:1000.

Results
No sensitisation was reported.

Comments
The study is inconclusive. A non-standardised test method was used and Freund's complete adjuvant was not applied.

Ref. : 7

2nd guinea pig sensitisation test

Guideline	:	none
Species/strain	:	Pirbright white guinea pigs
Group size	:	25 female, 15 test animal and 10 control
Test substance	:	Disperse red 15 (CI 60710)
Batch no	:	Not given (purity not stated in report)
Dose levels	:	1% aqueous solution
GLP	:	study not in compliance

The test animals received intra-cutaneous injections daily for 5 days with 3 intra-cutaneous injections (0.1 ml) with the test substance at 1% solution. Four weeks later intra-cutaneous challenge was carried out on the opposite flank using dilutions of the 1% solution at 1:10, 1:100, 1:500, and 1:1000.

Results
No positive reaction was seen.

Conclusion
The study is inconclusive as the method is not standardised and considered inadequate. Freund's Complete Adjuvant was not applied.

Ref. : 36

Human repeated insult patch test

Guideline	:	none
Species/strain	:	human volunteers
Group size	:	104 female volunteers at an age range of 18-65 years
Test substance	:	3 samples were prepared at a level of 3% in a vehicle composed of isopropanol, Tween 80, Natrosol, sodium sulphite and water
Batch no	:	Sample A 2181083 Sample B 3780284 Sample C 3421183 (Disperse Violet 1)
Dose	:	closed patch with Park Davies ready bandages and 3% concentration of test substance in vehicle
GLP	:	study not in compliance

0.1 ml of the test substance formulation was applied under closed patches to the infra-scapular region of the back, right or left of the midline. The patches were removed after 24 hours, and evaluation done 24 hours later and identical patches were reapplied. This treatment was repeated to give a total of 9 patch applications and readings. The last treatment was followed by a 14-day rest period.

The challenge patches were applied to test sites previously unexposed to the test formulation and remained under occlusion for 24 hours. Readings were carried out 48 and 72 hours after application (24 and 48 hours after patch removal).

Results
104 of 116 enrolled subjects completed the study.
Sample A : There was no evidence of sensitisation to this test material. One volunteer developed reactions during induction and challenge suggesting a possible pre-sensitisation to the test material.

Sample B: One subject developed a strong oedematous challenge reaction with some spreading suggestion definite sensitisation. Another developed a low-grade irritation reaction although sensitisation cannot be entirely ruled out, and further one volunteer developed the reaction suggestion pre-sensitisation.

Sample C: One individual developed reaction on challenge to 3421183 disperse violet 1suggestive of irritation although sensitisation cannot be entirely excluded, and another volunteer developed reactions suggesting pre-sensitisation.

Conclusion
In conclusion 1-3 of 104 subjects showed sensitisation in this human repeated insult patch test.

Ref. : 8

2nd test

Guideline	:	none
Species/strain	:	human volunteers
Group size	:	98 females
Test substance	:	Disperse violet 1, sample B 3421183
Batch no	:	not given (purity not stated in study report)
Dose	:	0,1 ml test substance under occlusive patch (Pack Davies Ready bandages)
GLP	:	study not in compliance

The test compound was formulated as a 3% preparation in a vehicle similar to the one used in the previous HRIPT.

For induction 0.1 ml of the test substance was applied under occlusion for 24 hours to the volar aspect of the right or left arm. Evaluation was done 24 hours after patch removal and identical patches re-applied. This treatment was repeated to give a total of 9 consecutive applications and readings. The last treatment was followed by a 11-day rest-period. At tenth application was inadvertently applied.
The challenge patches were applied to the opposite arm, and remained under occlusion for 24 hours. Readings were carried out 48 and 72 hours after application (24 and 48 hours after patch removal).

Results
98 of 104 volunteers completed the study. One subject developed reactions on challenge that required further testing to rule out sensitisation. This individual was re-challenged and developed erythematous reactions both on the back and on the arm, visible at both 48 and 72 hours.

Conclusion
There is some evidence of sensitisation to sample B, disperse violet 1 – 3421183, under the conditions employed in the study.

Ref. : 9

Photo-sensitisation

Guideline	:	none
Species/strain	:	Hartley albino guinea pigs
Group size	:	8 male and 8 female
Test substance	:	Disperse violet 1
Batch no	:	CN2121085, TSL no. 86-08
Dose	:	10% for induction and 5% for challenge in a vehicle composed of 80% DAE433 (40% dimethylacetamide, 30% acetone, 30% ethanol) and 20% physiological saline
GLP	:	study not in compliance

The minimal erythemal dose (MED) for UVA and UVB was determined in guinea pigs. A 150 watt xenon lamp was used to expose all animals during the study. The light scores emitted UVA (320-410 nm), UVB 280-320 nm), and visible light waves (410 nm and greater). The animals were shaved and depilated 1 day prior to induction and daily during induction and challenge. 1 ml of the test material was spread over a test site (diameter 1,8 cm) on the nuchal area on 4 consecutive days. This was repeated in 3 weeks. 1 hour after application the animals were irradiated with ½ MED of UVA light (1 week), respectively with 1 MED of UVB light (2. and 3. week). Freund's complete adjuvant injections in physiological saline at 1:1 were done on the first and third days of the second and third application week to 4 sites around the application site. Challenge was carried out on three different sites two weeks after the last induction treatment. Each site (1.8 cm diameter) was treated with 0.1 ml of the test material for 3 consecutive days. Determination of UVB photo-contact sensitisation was done on the left lumbar area by irradiating the animals with ½ MED UVB one-hour post application. UVA photo-contact sensitisation was checked on a site below by using ½ MED UVA one-hour post application. A third site remained unirradiated for determining contact sensitisation. All sites were inspected 24 hours after each application. Musk ambrette served as positive control in this study.

Results
The MED was 14 minutes for UVA and 90 seconds for UVB. There was no evidence of irritation at the test sites for the test substance, and it did not show any evidence of photo-allergic reaction in the guinea pigs.

Ref. : 10

2.6. Reproduction toxicity

2.6.1. One-generation reproduction toxicity

1,4-Diamino-anthraquinone was administered by gavage to pregnant female Sprague Dawley rats on day 6 through 15 of gestation at the dose levels of 10, 40 and 160 mg/kg/day body weight according to the OECD N°414. A control group was administered with the vehicle only, a 0.5 % aqueous swelling of Na-carboxymethylcellulose (CMC).

Pregnant animals were killed on day 20 of gestation ; visceral and skeletal malformations were recorded on the foetuses. No female mortality was recorded during the study. However, significant maternal toxicity was observed : decrease of body weight gain and food consumption from day 6 to 11. At the external examination, six foetuses of one dam in the high dose group had severe general subcutaneous oedema in connection with discoloured placentae. At the skeletal examinations, the incidence of lumbar rudimentary ribs slightly higher in foetuses of all dosed groups was noted. At the visceral examination, severe general subcutaneous oedema with corresponding hydrocephalus and diaphragmatic herniation in foetuses of high dose group were observed. These observations were not considered by investigators to be a teratogenic effect but secondary to maternal toxicity. None of the effects recorded were considered as teratogenic.
Under the experimental conditions adopted, 1,4-Diamino-anthraquinone reveal none teratogenic or embryo-toxic effects, the No-Observable-Adverse-Effect-Level of the test product was established at 40 mg/kg bw.

Ref. : 30

The sub-chronic toxicity, reproductive effects and mutagenic potential (dominant lethal) of a hair dye formulation containing 34.9 % - 41.4 % 1,4-Diamino-anthraquinone (C64) and 2.22 % - 3.2 % 1-amino-4-hydroxyanthraquinone (C61) were evaluated in groups of 40 male and 45 or 55 female Sprague Dawley rats. They were fed with the preparation at the dietary levels of 0.02, 0.06 and 0.2 % for up to six months. Clinical chemistry, haematology and histopathology studies were performed in subgroups of 10 males and 10 females after 13 and 27 weeks. After 15 weeks, 25 females per group were mated to untreated males in a teratology study. After 19 weeks, 20 males per group were removed from the test diets and mated on two separate occasions with two untreated virgin females in a dominant lethal mutagenicity study. Ten females from the mid and high levels were removed from the test diet at the same time. These males and females remained untreated until they were killed at the end of the study, together with animals that had been maintained on the test diets for the entire period.

One female mid dose level died during the study and three rats (two high dose, one low dose), were sacrificed in a moribund condition. Discoloured violet urine was observed in all dye treated rats. The high dose level males gained significantly less weight than the controls during the onset of the study and comparatively to the high level females. Hair loss was recorded in high dose group female and in the control group. Regrowth was seen following cessation of treatment. Statistically significant increase in liver and kidney weights were observed in various treated groups. Slight increase in cholesterol in high dose males and treated females was noted. Histomorphologic alterations were observed in the liver at the mid and high level and in the kidney at the high dose level. Hepatic lesions seen at the high level consisted of hepatocytomegaly, principally in the centrilobular area, minimal to moderate deposition of pigment, Kupffer cell proliferation and necrosis and vacuolation of hepatocytes. Renal changes observed at the high dose level consisted of minimal brown pigment in the tubules and changes characteristic of chronic nephropathy were more frequent and more severe than those seen in control rats. Renal and hepatic effects were generally more marked in males. Concerning teratology study, one malformed foetus was found in each of the control and treated groups, these observations were not dose related and not considered to be treatment related by investigators. The observed external and skeletal variations were similar across groups. There were statistically significant increases of the numbers of dams with resorptions at the low and mid level in the second mating of the dominant lethal study. However, there were no increases in non-viable

foetuses at the high dose level, nor statistically significant effects at any dosage level in the first mating.

Based on these results, the test preparation was considered by investigators not teratogenic or foeto-toxic. No dominant lethal effect was retained for the preparation tested among these data.

Ref. : 31

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Percutaneous absorption *in vitro*

Guideline	:	none available
Tissue	:	1 mm full thickness skin of male castrated domestic pigs
Method	:	flow through diffusion cells made of Teflon.
Test substance	:	Disperse violet A (CI 61100)
Batch no	:	not given
Dose levels	:	0.1 g/cm^2 of 1% concentration of test substance in koleston 2000
GLP	:	study not in compliance

The test substance was incorporated at 1% in a hair dye formulation and applied without peroxide. 0.1 g of the formulation was applied pr. cm^2 of skin on a surface of 9-10 cm^2 for 30 minutes. Residues were removed by a spatula and the skin was washed with warm water and neutral shampoo. Percutaneous absorption was determined after 72 hours. The receptor fluid circulating in the system was a phosphate buffer containing antibiotics, and it was collected with 8-16 hours interval. After solid phase extraction the amount of the test substance in the receptor solutions was measured by HPLC.

Results
In 6 cells absorption was followed for 72 hours after 30 minutes application of a 1% preparation. A total of 141.5±35 ng/cm^2 was absorbed. In further 3 cells absorption was measured after an application time of 16 hours. The total absorption was 145±27 ng/cm^2. After the end of the study the amount of test material present in the skin was measured and varied between 60 and 250 ng/cm^2 in the cells exposed for 30 minutes, and between 850 and 1800 ng/cm^2 in the cells exposed for 16 hours.
The mean percutaneous absorption rate is calculated to be 0.014%.

Comment
The quantities of the test substance applied are very low and able to distort the results obtained. Mass balance is not determined.

Ref. : 12, 13, 14

2.8. Mutagenicity/Genotoxicity

1,4 Diamino-anthraquinone (Colipa C-64) is always contaminated with 1-Hydroxy-4-amino-anthraquinone (Colipa C-61)

C-64	95.6 – 97.3 % CAS No. 128-95-0
C-61	2.7 – 4.4%

2.8.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

The study comprises 90 different anthraquinones derivatives, including C-64. The purity of the chemical has not been indicated.

A *Salmonella*/microsome test was applied according to the 1970 practices. Colipa C-64 resulted positive at all concentrations tested, from 100 to 2000 μg/plate ± S-9 mix (strain TA1538 and TA98).

The chemical, in spite of the inadequacy of the technical details present, is considered mutagenic in the bacterial gene mutation assay.

Ref. : 18

Bacterial gene mutation assay

Guideline	:	None
Species/Strain	:	*Salmonella typhimurium* TA1535, TA1537, and TA 1538 (± S9 mix)
Replicate	:	2 independent experiments. Data are reported only for one experiment, with 3 plates.
Test substance	:	1-4-Diaminoanthraquinone, no purity was indicated (purple coloured powder)
Batch No	:	Not indicated
Concentrations	:	4 doses: 5, 50, 500, 5000 μg/well
GLP	:	None

The study cannot be accepted for the evaluation due to some technical inadequacies, such as the absence of indication on purity, the absence of a replicate of the experiment, the type of the strains used, not including those sensitive.

Ref. : 19

Bacterial gene mutation assay

The study could not be evaluated because the induction of mutation on *Salmonella typhimurium* cells was investigated only in the absence of a metabolic activation system. The study was published in 1978.

Ref. : 20

Bacterial gene mutation assay

The study could not be evaluated due to the absence of several technical details, including numerical results. The study was published in 1979.

Ref. : 21

Bacterial gene mutation assay

Guideline	:	OECD and EPA
Species/Strain	:	*Salmonella typhimurium strains* TA1535, TA1537, TA 1538, TA98, TA100 (± S9 mix) obtained by rat liver induced by Aroclor 1254
Replicates	:	2 independent experiments
Test substance	:	C64 disperse violet 1
Batch No	:	7634/124 (black powder) lot #902873
Purity	:	not stated
Concentrations	:	5, 16.7, 50, 167, 333 and 500 μg/plate (± S9 mix) DMSO 0.167, 0.5, 1.67, 5, 16.7 and 50 μg/plate (± S9 mix) DMSO
GLP	:	FDA, EPA
Positive controls	:	Sodium Azide (TA1535, TA100), 9-AA (1537), 2NF (TA1538, TA98); 2-Anthramine (+S9 mix)

Results
Dose dependent, statistically significant, increases in the no. of revertants were observed in all tester strains (2.1 to 130 fold) in the presence of S9 mix and in the strains TA1535, TA1537, TA1538 and TA100 in the absence of S9 mix.
The positive controls gave the results expected.
The positive results were confirmed in the second experiment.

The Colipa C-64 was found positive in the *Salmonella* plate incorporation assay and it was considered mutagen for the induction of gene mutation in bacterial cells.

Ref. : 22

Bacterial gene mutation assay

Guideline	:	None
Species/Strain	:	*Salmonella typhimurium* strains TA1535, TA1537, and TA1538 (± S9 mix)
Replicate	:	2 independent experiments. The data of 1 experiment are reported, with 3 plates
Test substance	:	1-amino-4-hydroxyanthraquinone, Disperse red 15 (brown powder)
Batch	:	not indicated
Concentrations	:	10, 100, 1000, 10,000 μg/plate
GLP	:	None

Results
The study cannot be accepted for the evaluation due to some technical inadequacies, such as the absence of a certificate of analysis and no. of batch, the lack of results, the absence of the most sensitive strains of bacterial cells.

Ref. : 37

Bacterial gene mutation assay

The study could not be evaluated because the induction of gene mutation in the *E.coli* cells was investigated only in the absence of a metabolic activation system. The study was performed in 1974.

Ref. : 38

***Saccharomyces cerevisiae* gene mutation assay**

The study could not be evaluated because the induction of mutation on *S.cerevisiae* cells was investigated only in the absence of a metabolic activation system. The study was published in 1978.

Ref. : 20, 23

Bacterial gene mutation assay

The study could not be evaluated because the induction of gene mutation in the *Escherichia coli* cells was investigated only in the absence of a metabolic activation system. The study was performed in 1976.

Ref. : 24

Bacteriophage T4D mutagenesis

The study refers to a non standardized organism, although the results indicated a strong mutagenic effect.

Ref. : 25

Chromosome aberrations in Chinese Hamster (CHL) lung cells assay

Data obtained from a book published by M.ISHIDATE in 1988 (experiments performed in his own laboratory) showed a strong positive effect in the absence of a metabolic activation at all concentrations 0.0625, 0.125, 0.25 mg/ml after 24 hours and 48 hours (only 0.25 mg/ml, tested on DMSO.
No technical details are presented.

The Colipa C-64 substance was found positive for the induction of all types of chromosome aberrations on Chinese Hamster lung cells in the absence of a metabolic activation system.

Ref. : 26

Chromosome aberration assay on human lymphocytes

Guideline	:	According to the literature
Species/Strain	:	human peripheral blood lymphocytes with and without metabolic activation (rat livers Aroclor treated)
Replicate	:	Duplicate treatment

Test substance	:	1-4-Diaminoanthraquinone (C-64) purity not stated
Batch No	:	14.05.90
Concentrations	:	30, 300 and 1000 μg/ml -S9 mix (24h) 100, 1000, 3000 μg/ml +S9 mix (2h) OECD
GLP	:	OECD
Positive controls	:	CPA and MC
Negative control	:	Medium

Results
The Colipa C-64 substance was shown to induce a statistically significant dose related increase in the chromosome aberrations in human lymphocytes cells assay.
The positive controls gave the expected results.
This substance, although its purity is not indicated, is able to induce cytogenetic effects in human lymphocytes treated *in vitro* in the presence and absence of chromosome aberrations.

Ref. : 27

Chromosome aberrations assay on CHO cells

Guideline	:	US-EPA
Species/Strain	:	Chinese Hamster Ovary cells (CHO-K1) from ATCC with and without a metabolic activation system (rat livers Aroclor treated)
Replicate	:	Two independent cultures
Test substance	:	1-4-Diaminoanthraquinone (C-64)
Purity	:	36.5% The substance contains 3.9% of 1-amino-4-hydroxyanthraquinone (C-61) and 1.1% of other three anthraquinone substitutes. The substance contains 51.2% of uncoloured material not identified
Batch No	:	005 from Compton & Knowles Corporation Reading, Pa, USA (C-64 Disperse Violet 1)
Concentrations	:	1, 5, 10, 50, 100 μg/ml in the presence (44) and absence (204) of S9 mix;
GLP	:	FDA
Positive controls	:	MMC and CP
Negative control	:	DMSO

Results
The Colipa C-64 substance was shown to induce a statistically significant dose-dependent increase in the chromosome aberrations in CHO-K1 mammalian cells treated *in vitro* in the presence and absence of a metabolic activation system.
The positive controls gave the expected results.
This substance is of 36.5% of purity and includes 5% of other anthraquinones and 51.2% of undefined material.
The test substance is able to induce cytogenetic effects in mammalian cells grown *in vitro*, in all methodological conditions.

Ref. : 28

2.8.2. Mutagenicity / Genotoxicity *in Vivo*

Mouse bone marrow micronucleus test

Guideline	:	None (literature)
Species/Strain	:	Adult NMR1 male & female mice
Group size	:	5 males + 5 females
Test substance	:	Diaminoanthraquinone (C-64)in physiological saline
Purity	:	not stated
Batch No	:	Ba. 14.5.90
Dose levels	:	1,500 mg/kg p.o. (Maximum Tolerated Dose) 24, 48 and 72 hours
GLP	:	Declaration of QAU
Positive control	:	DMBA

Results
The substance, Colipa C-64, of unknown purity, was tested at a supposed Maximum Tolerated Dose (no data are included) on mice (5 males and 5 females) treated for 24, 48 and 72 hours by o.s. for the induction of micronucleated cells in the bone marrow.
9,10-dimethyl-1,2-benzanthracene was employed as a control.
There was no indication of an induced increase of Micronuclei in all three treated conditions; the positive control, analysed after 48h, showed positive results. The negative control was analysed only at 24h time.
There was not a statistically significant decrease of the ratio PE/NE in all three conditions of treatment.

Conclusions about the interpretation of these results cannot be drawn because :

- The absence of a toxicity test showing that the dose 1500 mg/kg was the maximum tolerated dose
- The negative control was evaluated only at the time 24 hours
- Statistical analysis of the PE/NE ratio is not included. The data show some variability for some individual animal
- The purity of the substance employed in the test is not indicated

The study could be considered inadequate for the evaluation of the mutagenic potential of this substance *in vivo*.

Ref. : 29

Conclusions

The evaluation of the mutagenic potential of C61 is not possible at present, since no adequate data have been produced.

The Substance C-64 is always contaminated with other anthraquinones substitutes. In some mutagenicity/genotoxicity studies impurities over 50% have been reported to be present in the material employed for the tests.

Colipa C-64 has been tested by means of the following assays :

Bacterial gene mutation
Yeast gene mutation
Mammalian Cytogenetic *in vitro*
Mouse bone marrow
Other assays

Only a few of these assays have been accepted and fully evaluated, the remaining being inadequate, as stated in their presentation. The following conclusions can be drawn for final evaluation :

The substance, of unknown purity, has been found: positive in the Bacterial gene mutation assay (22), positive for the induction of chromosome aberration induction in Chinese Hamster Ovary Cells (28), human lymphocytes (27), Chinese Hamster Lung cells (26).

The substance C-64 is considered mutagenic *in vitro* for the induction of gene mutation in bacterial cells and chromosome aberrations in mammalian cells

2.9. Carcinogenicity

/

2.10. Special investigations

/

2.11. Safety evaluation

CALCULATION OF THE MARGIN OF SAFETY

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

Highest penetration	**PA (μg/cm2)**	**=**	
Typical body weight of human		**=**	**60 kg**
Exposed area (scalp)		**=**	**700 cm2**
Systemic exposure	**PA x 700 cm2**	**=**	**mg**
Systemic exposure dose (SED)	**PA x 700/ 60 x**	**=**	**mg/kg bw**
No observed adverse effect level (mg/kg) (species, route of application)	**NOAEL**	**=**	**mg/kg bw**

Margin of Safety	**NOAEL / SED**	**=**

2.12. Conclusions

Disperse Violet 1 (C 64) is a compound impurified with Disperse Red 15 (C 61) and intended to be used in colour setting lotions at a maximal concentration of 2 %. Basing on a usage volume of 35 ml of hair dye preparation per application, the maximum amount applied would be close to 700 mg.

* Disperse Violet 1 is neither irritant to the skin nor to the eye. After repeated topical applications of Disperse Violet 1, no adverse effects were noted. No data is available on irritant potential of Disperse Red 15 (C61). In view of the low concentration, this may not be important.

* The allergenicity of the compounds cannot be assessed due to inadequate data.

* From the sub-chronic studies conducted by oral route in the rat, a NOAEL of 1.21 mg/kg/day for Disperse Violet 1 and of 0.19 mg/kg/day for Disperse Red 15 was obtained.

* The percutaneous absorption study is considered inadequate

* The evaluation of the mutagenic potential of Disperse Violet 1 is not possible at present since no adequate data has been produced. Disperse Red 15 of unknown purity has been found positive in the bacterial gene mutation assay and for the induction of chromosome aberration in

two mammalian cell lines grown *in vitro* and on human lymphocytes. The substance is considered an *in vitro* mutagen.

An *ex vivo* study has been conducted on pig skin showing an absorption rate of 1,4-Diamino-anthraquinone amounted 0.014 % after 30 minutes contact to the skin.

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, an allergenicity and an *in vitro/in vivo* percutaneous absorption study should be performed in accordance with the SCCNFP Notes of Guidance as well as an in vivo genotoxicity/mutagenicity study according to OECD Guidelines.

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