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

CONCERNING

ACID ORANGE 7

COLIPA n° C15

adopted by the SCCNFP on 23 April 2004
by means of the written procedure

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 Acid Orange 7 safe for use in cosmetic products as a hair dye ingredient?

* 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

Acid Orange 7 is listed as CI 15510 in Annex IV, part 1 – list of colouring agents allowed for use in cosmetic products – to Directive 76/768/EEC on cosmetic products; field of application 2: colouring agents allowed in all cosmetic products except those intended to be applied in the vicinity of the eyes, in particular eye make-up and eye make-up remover.

2.1.1. Primary name

Acid Orange 7 (INCI)

2.1.2. Chemical names

Sodium 4-[(2-hydroxy-1-naphthyl)azo]benzene sulfonate (EU inventory)
4-[(2-hydroxy-1-naphthyl)azo]benzenesulfonic acid, monosodium salt,

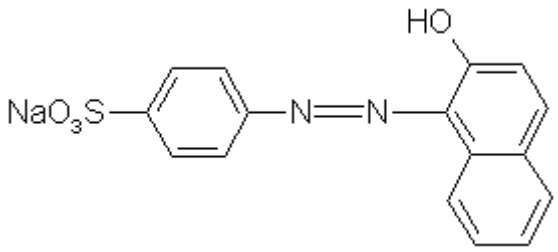
2.1.3. Trade names and abbreviations

COLIPA n°	:	C 15
Trade names	:	Orange 205; Acid Orange 7 monosodium salt; Orange II
Other names	:	D&C Orange 4, Acid Orange 7

2.1.4. CAS / EINECS / COLOUR INDEX number

CAS	:	633-96-5
EINECS	:	211-199-0
Colour Index	:	CI 15510

2.1.5. Structural formula



2.1.6. Empirical formula

Emp. Formula	:	$C_{16}H_{11}N_2NaO_4S$
Mol weight	:	350.3

2.1.7. Purity, composition and substance codes

Substance code : /

Batches used : Chemical Identification of Acid Orange 7 (Batch No. 2097AF, Lot No. AJ3559, Purity approx. 90%) was performed by UV-Vis, IR, NMR and MS. The sample used for HPLC analysis is identified as D&C Orange 4 120.8

Purity	:	90% (total colour content)
Relative chromatographic purity (HPLC - UV/VIS peak area method)	:	99-100% at 210 nm, 254 nm, 480 nm)
Sulfate ash	:	20-25%
Water content	:	0-5%

Impurities		
2-naphthol	:	0-0.1%
Sulfanilic acid	:	0-0.1%
4-4-(Diazoamino)dibenzenesulfonic acid	:	< 0.1%
Lead	:	20 ppm
Arsenic	:	3 ppm
Mercury	:	1 ppm

Batch Comparison

Lot No	R0073770	AJ3559	AK5453	AL1478
Batch No	0201212137	2097 AF		DC04/7
		FDA certified	FDA certified	FDA certified
Total color	99% (HPLC)	90%	91%	96%
Volatile matter		6.7%		2.7%
NaCl		2.7%		1.2%
Na_2SO_4		0.05		0.05
Water insoluble matter		0.10%		0.03%
2-Naphthol	0.055%	0.06%		0.02%
Sulfanilic acid	0.012%	0.07%		0.03%
Mercury	<10 ppm	PT		PT
		PT		PT
		PT		PT

2.1.8. Physical properties

Appearance	:	Orange powder, odourless
Melting point	:	decomposition at 164°C
Boiling point	:	/
Density	:	/
Rel. vap. dens.	:	/
Vapour Press.	:	/
Log P_{ow}	:	1.4 (HPLC method)

2.1.9. Solubility

Water	:	11% at 30°C
Saline	:	2%
DMSO	:	10%
Formulation	:	2%

2.1.10 Stability

Storage : light and humidity protected at room temperature
Aqueous solution stable ≥72 hours

General comments on analytical and physico-chemical characterisation

* Absolute content of Acid Orange 7 has not been reported for any batch of the test material
* Stability of the test material in prototype formulation(s) is not reported.

2.2. Function and uses

Acid Orange 7 is intended for use in non-oxidative hair dye formulations as a direct dye at a maximum concentration of 0.5%

TOXICOLOGICAL CHARACTERISATION

Part of the basic toxicological data was presented in form of original articles mostly published in well reputated and peer reviewed journals. As the substance is already in use for decades part of the toxicological data was originated between 1950 and 1980; another part between 1981 and 2000, on mutagenicity some in 2003.

Moreover the applicant of the dossier carried out a literature search using the ChemI Plus-system, a well reputated search system including most of the acknowledged data bases as MEDLINE, TOXNET NLM Gateway etc. A statement is given that all hits of relevance for risk assessment of Acid Orange 7 were included in the presented files.

2.3. Toxicity

2.3.1. Acute oral toxicity

LD_{50} (rats)	> 10.000 mg/kg/bw.
LD_{50} (♀ mice)	> 10.000 mg/kg/bw.

Ref.: 1

LD_{50} (♀ +♂ rats) > 10.000 mg/kg/bw.

Ref.: 2, 3, 4

LD_{50} (♀ +♂ rats) 11.300 mg/kg/bw.

Ref.: 5

Dogs 1.000 mg/kg/bw.

Ref.: 5

Conclusion
The test substance is of very low acute toxicity.

2.3.2. Acute dermal toxicity

No data

2.3.3. Acute inhalation toxicity

No data

2.3.4. Repeated dose oral toxicity

14-days oral toxicity (range-finding study 1)

Guideline	:	/
Species/strain	:	SPF rats, strain HanIbm: WIST
Group size	:	5/5 (each concentration)
Test substance	:	Acid Orange 7 in aqueous sol. + 1 % CMC
Batch	:	No. 2097AF, Lot No. AJ 3559; purity 90 %
Dosages	:	0, 10, 60, 100, 1000 mg/kg/bw.; by oral gavage
GLP	:	/

40 rats (20 males and 20 females, strain HanIbm:WIST, SPF) were used for this assay. The body weights of the animals ranged between 88-114 g for males and between 77-104 for females at the beginning of the study.

The test material (purity 90 %, Batch 2097AF; Lot no. AJ3559) was homogenized in bi-distilled water containing 1 % CMC.
The following dose levels were tested in this study: 0, 10, 60, 100, 1000 mg/kg b.w..
The animals (5 males, 5 females, each concentration) were treated by oral gavage, once daily, 7 days per week for 14 days.
Clinical signs, food consumption and body weights were recovered periodically during pretest, and treatment period. All animals were killed, necropsied and examined post mortem.

Results
On the basis of the results obtained in the 14-day dose range finding study a proposal of dose levels for 90 day subchronic toxicity study is not possible. Therefore, an additional 14-day range finding study with the following dose levels should be envisaged:
0, 2.5, 5, 10 mg/kg bw.

Ref.: 12

14 days oral toxicity (range finding study 2)

Guideline	:	/
Species/strain	:	SPF rats, strain HanIbm: WIST
Group size	:	5/5 (each concentration)
Test substance	:	Acid Orange 7 in aqueous sol. + 1 % CMC
Batch	:	No. 2097AF, Lot No. AJ 3559; purity 90 %
Dosages	:	0, 2.5, 5, 10 mg/kg/bw; by oral gavage
GLP	:	/

40 rats (20 males and 20 females, strain HanIbm:WIST, SPF) were used for this assay. The body weights of the animals ranged between 94-118 g for males and between 85-106 for females at the beginning of the study.

The test material (purity 90 %, Batch 2097AF; Lot no AJ3559) was homogenized in bi-distilled water containing 1 % CMC.
The following dose levels were tested in this study: 0, 2.5, 5, 10 mg/kg bw.
The animals (5 males, 5 females, each concentration) were treated by oral gavage, once daily, 7 days per week for 14 days.
Clinical signs, food consumption and body weights were recovered periodically during pretest, and treatment period. At the end of the dosing, blood samples were withdrawn for haematology and plasma chemistry analyses. All animals were killed, necropsied and examined post mortem. Histological examinations were performed on organs and tissues from all animals.

Results
On the basis of the results obtained in the 14-day dose range finding study the following dose levels for the 90 day subchronic study were proposed:
0, 2.5, 5, 10 mg/kg bw.

Ref.: 13

2.3.5 Repeated dose dermal toxicity

No data

2.3.6. Repeated dose inhalation toxicity

No data

2.3.7. Subchronic oral toxicity

13-weeks oral toxicity

Guideline	:	OECD 408 (1998)
Species/strain	:	SPF rats, strain HanIbm: WIST
Group size	:	10/10 (each concentration)
Test substance	:	Acid Orange 7 in aqueous sol. + 1 % CMC
Batch	:	No. 2097AF, Lot No. AJ 3559; purity 90 %
Dosages	:	0, 2.5, 5, 10 mg/kg/bw; by oral gavage
GLP	:	in compliance

80 rats (40 males and 40 females, strain WISTAR, HanIbm:WIST (SPF)) were used for this assay. The body weight of the animals in this test ranged between 123-163 grams (mean 141 gram) for males and between 98-143 grams (mean 123 grams) for females.

The test was performed according to the following guidelines: Repeated dose 90-day oral toxicity study in rodents" OECD Guideline for the testing of chemicals, Section 4, Health Effects, Number 408, adopted 21st September 1998.

The test material (purity 90 %, Batch 2097AF; Lot no. AJ3559) was homogenized in bi-distilled water containing 1% CMC. According to the result of 14 day dose range finding study (RCC 746460) the following concentrations were used for the 90 day oral gavage study: 0, 2.5, 5, 10 mg/kg bw/day The animals (10 males, 10 females, each concentration) were treated by oral gavage, once daily, 7 days per week for at least 13 weeks.

Results
Changes in haematology were evident in males treated with 5 and 10 mg/kg/day. Changes in haematological parameters like increased methemoglobin levels in males up to 2.5 mg/kg bw and females up to 5 mg/kg bw, decreased hemoglobin levels in males (5 and 10 mg/kg/day) increased reticulocyte counts (relative and absolute) in all test article treated males and a general shift towards high fluorescent reticulocytes in high dosed males. The changes noted in methemoglobin levels and reticulocte counts in males treated with 2.5 mg/kg/day were within the upper levels of the historical control data.
When compared with similarly high values at 5 mg/kg/day, there was no correlation to pathomorphological (extramedullary hemopoiesis in the spleen) or other haematological parameters at 2.5 mg/kg/day.
In males treated with 10 mg/kg/day, increased organ/body weight ratio in the spleen was noted, which correlated to microscopic findings (extramedullary hemopoiesis in the spleen in all animals treated with 5-10 mg/kg/day.
No test article related macroscopic findings were observed during necropsy.
With regard to the results obtained this, especially to changes in haematological parameters of males, the No-Observable-Adverse-Effect-Level (NOAEL) of Acid Orange 7 [D&C Orange 4 (C.I. 15510)] was considered to be in the general range around 2.5 mg/kg body weight/day. This dose led to borderline effects on met-hemoglobin and reticulocytes counts without concurrent effects on the spleen. The NOAEL for females could be set to 2.5 mg/kg/body weight/ day.

Ref.: 14

2.3.8. Sub-chronic dermal toxicity

3 animals each group (rabbits) were used for this assay.

The test material (FDA certified material) was applied to the depilated skin of rabbits. Daily applications were made to intact and abraded skin for 21 days and to intact skin for 90 days. The colour was applied at two concentrations 0.1 and 1.0 %, dissolved in water as well as USP White Ointment. Control groups were treated with respective media.

Results
There was no mortality nor any evidence of systemic toxicity. Haematological values, growth responses and urinary components were normal. Gross autopsies disclosed no dose-related findings.

Ref.: 15

2.3.9. Sub-chronic inhalation toxicity

No data

2.3.10. Chronic toxicity

18-month dermal toxicity (Literature data)

200 mice (100 male and 100 female) served as controls and 100 mice were assigned to the test article treatment (Swiss Webster mice).
Initial weights of the animals ranged from 17-25 g.
FDA certified colour was used.
The colour was dissolved in distilled water. Mice were painted once weekly with 0.1 ml of the solution or suspension containing 1 % of dye on actual pigment basis to a depilated area of 6 cm^2.
Survival, body weight and palpable growths were followed for 18 month. Each mouse was observed daily for behaviour, survival and visible growth.
All mice were necropsied after they died or were sacrificed. Organs were fixed in 10 % formalin solution after recording any gross pathological findings. Histological examinations were carried out on all tumours and all visibly abnormal organs.

Results
The authors conclude that no adverse reactions or pathological changes were observed following weekly dermal applications of D&C Orange 4.

Ref.: 16, 17

Comment
The study can not be regarded as sufficient for evaluation.

For additional studies see also 2.9.

2.4. Irritation & corrosivity

2.4.1. Irritation (skin)

Guideline	:	/
Species/strain	:	albino rabbits (1.5 – 2.0 kg)
Size	:	4 males
Test item	:	Orange II.
Batch no.	:	/
Purity	:	/
Dose	:	100 mg
GLP	:	/

No experimental data are presented. The protocol was following the modified method of Draize.(Ref. 6). Results were published in the literature in 1981 (Ref. 7)

Result
Orange 4 produced very slight redness of abraded skin in some of the rabbits when observed at only 24 hours after application. The authors concluded that this was false positive reaction. Under the conditions in this experiment the material is classified as non-irritant to the skin.

Ref.: 6, 7

Comment
Assessment of skin irritation is not complying with the actual standards; nevertheless, there is no reason to ask for a repetition of the skin irritation study.

2.4.2. Irritation (mucous membranes)

Primary eye irritation, undiluted test compound

Guideline	:	/
Species/strain	:	rabbit
Group size	:	3 males
Test substance	:	Orange II.
Purity	:	/
Batch no	:	/
Dose level	:	100 mg
GLP	:	/

No experimental data are presented. Protocol and results were published in the literature in 1981 (Ref. 7)

Result
Based on the result of the experiment the authors conclude that Orange II can be classified as non-irritant to the eyes.

Ref.: 7

Primary eye irritation, diluted test compound

Guideline	:	/
Species/strain	:	rabbit
Group size	:	6
Test substance	:	Orange II.
Purity	:	/
Batch no	:	/
Dose level	:	10 % solution, twice daily, 5 days/week, 4 weeks
GLP	:	/

No experimental data are presented. Protocol and results were published by Burnett and Opdyke in 1971, and were briefly cited in Ref. 7.

Result
Orange II is not-irritant to the eyes in this test.

Ref.: 7

Comments
Assessment of eye irritation is not complying with the actual standards; nevertheless, there is no reason to ask for a repetition of the eye irritation study.

2.5. Sensitisation

Magnusson & Kligman Maximisation test

Guideline	:	OECD 406 (1981)
Species/strain	:	Dunkin-Hartley guinea pig
Group size	:	20 females in test group, 10 females in control group
Observ. Period	:	25 days
Test substance	:	Orange 205
Purity	:	> 85%
Batch no	:	/
GLP	:	in compliance

A pretest was performed in order to assure an optimum technical application procedure.
The main study was performed as follows:

Induction: intradermal induction of sensitization (day 1) in the test group was performed with Freund´s Complete Adjuvant (FCA) and physiological saline (1:1), test item at 25 % in liquid paraffin, 25 % dilution of the test item in FCA plus liquid paraffin; ratio 1:1. One week later, the epidermal induction of sensitisation was conducted under occlusion with the test item at 50 % in paraffin for 48 hours.

Challenge: two weeks after topical induction, the challenge was performed by application of the test item at 10 % and 5 % in liquid paraffin under occlusive patch for 24 hours at a different part of the skin. Cutaneous reactions were evaluated at 24 and 48 hours removal of the dressings.

Result
After challenge no skin reactions were observed. Therefore, based on the result in this adjuvant test in guinea pigs the test article was classified as non-sensitizer.

Ref.: 10

Local Lymph Node Assay

Guideline	:	OECD 429 (2002)
Species/strain	:	Mice CBA/J
Group size	:	5 females per group
Test substance	:	D&C Orange 4
Batch No.	:	0201212137ICM Barrier, sample no R0073770
Purity	:	> 99.2 %
Concentrations	:	a) 0.3, 1, 3, 9 % (w/v) in DMSO b) 0.3, 1, 3 and 5.4 % (w/v) in water/acetone (1:1) mixed with olive oil (3:1)
GLP	:	in compliance

On three consecutive days, 25 µl of test item, vehicle and positive control were applied topically to the dorsal surface of each ear lobe. 5 days after first application [^{3}H]methylthymidine was intravenously injected into a tail vein. 5 hours later mice were sacrificed by carbon dioxide inhalation and the draining auricular lymph nodes taken and weighed. Single cell suspension was prepared for each animal. Cells were washed with PBS and precipitated with 5 % trichloro-acetic acid (TCA). 18 hours later the pellets were suspended in TCA and transferred into the scintillation cocktail. The proliferation capacity of lymph node cells was determined by the incorporation of [^{3}H]-methylthymidine. A test item is regarded as a sensitizer in the LLNA if the exposure to at least one concentration of the test item resulted in an incorporation of ^{3}HTdR at least 3-fold or greater than that recorded in control mice, as indicated by the stimulation index (S.I.)

Result
Based on the result in this LLNA in mice, Orange 4 is not a skin sensitizer under defined experimental conditions in the two vehicles tested.

Ref.: 11

2.6. Reproduction and Developmental Toxicity; Teratogenicity

Dose Range Finding Study for effects on Embryo-Foetal Development

Guideline	:	/
Species/strain	:	SPF rats, strain HanIbm: WIST
Group size	:	20 mated rats
Test substance	:	Acid Orange 7 in aqueous sol. + 1 % CMC
Batch	:	No. 2097AF, Lot No. AJ 3559; purity 90 %
Dosages	:	0, 100, 300, 1000 mg/kg/bw; by oral gavage (day 6 to 17 p.c.)
GLP	:	/

20 mated females, 5 per group (strain rat HanIbm:WIST, SPF) were used for this assay. The body weights of the animals ranged between 199-233 grams beginning of the study.
Test material (purity 90 %; Batch 2097AF, Lot no. AJ3559) was dissolved in bi-distilled water containing 1 % carboxymethylcellulose sodium salt.
20 mated females rats were treated orally by gavage with a single dose of the test article once daily from day 6 through to day 17 post coitum at dose levels of:
0, 100, 300, 1000 mg/kg bw.
A standard dose volume of 10 ml/kg bw with a daily adjustment to the actual body weight was used. Control animals were dosed with the vehicle alone.
Females were sacrificed on day 21 post coitum and the foetuses were removed by Caesarean section.

Results
With the exception of a slight reduced mean foetal body weight at 1000 mg/kg, no test article related differences were noted amongst the control group and any dose group. During external examination of foetuses no abnormal findings were noted in any group.
Because of the distinct effects on the spleen weight of dams already at a dose level of 100 mg/kg bw/day, suitable dose levels for the main study for effects on embryo-foetal development in the rat could not be established.

Ref.: 18

Study for the Effects on Embryo-Foetal Development

Guideline	:	OECD 414 (1981)
Species/strain	:	SPF rats, strain HanIbm: WIST
Group size	:	22 mated rats (each concentration)
Test substance	:	Acid Orange 7 in aqueous sol. + 1 % CMC
Batch	:	No. 2097AF, Lot No. AJ 3559; purity 90 %
Dosages	:	0, 5, 40, 320 mg/kg/bw; by oral gavage (day 6 to 17 p.c.)
GLP	:	in compliance

88 mated female rats at the age at pairing of minimum 11 weeks, 22 per group, (strain WISTAR, HanIbm:WIST [SPF]) were used for this assay. The body weight of the animals in this test was between 169-244 grams.

The test was performed according to the following guidelines:
Recommendation of the Commission of the European Communities, No. III/3387/93, guideline prepared within the International Conference on Harmonisation (ICH), Washington (DC), June 23, 1993 and "Teratology"; OECD Guidelines for the Testing of Chemicals, Section 4, Health Effects, No. 414, May 12, 1981.

The study was conducted in compliance with OECD Principles of Good Laboratory Practice, as revised 1997 [C(97) 186/Final].

The test material (purity 90 %, Batch 2097AF, Lot no. AJ3559) was homogenized in bi-distilled water containing 1 % CMC. The mixture of the test article and vehicle were prepared daily before administration. According to the result of dose range finding studies (RCC 733950 and RCC 746460) the following concentrations were used for the study: 0, 5, 40, 320 mg/kg bw/day. The animals (22 mated female rats, each concentration) were treated by oral gavage, once daily from day 6 through to day 17 post coitum (last treatment).

A standard dose volume of 10 ml/kg bw was used. Females were sacrificed on day 21 post coitum and the foetuses were removed after Caesarean section. The examination of the dams and foetuses was performed in accordance with international recommendations.

Results
Based on the result, the No-Observable-Adverse-Effect-Level (NOAEL) of Acid Orange 7 [D&C Orange 4 (C.I. 15510)] was considered to be 5 mg/kg body weight/day for the maternal organism and 320 mg/kg body weight for the foetal organism.

Ref.: 19

2.7. Toxicokinetics (incl. Percutaneous Absorption)

Percutaneous absorption study

Guideline	:	OECD 428 (1999)
Test system	:	pig ear skin (400-500 μm), 12 samples, skin integrity checked by conductivity measurement
Contact time	:	30 minutes then washing with a shampoo, diffusion monitored during 24 hours
Test substance	:	D&C orange 4 used at 0.5 % in a hair dye gel formulation batch:

		T1E20000642 (composition not stated)
Control	:	"neutral gel" (composition not stated)
Purity	:	90 % (raw material)
Batch no	:	2097AF (raw material) ,
Application	:	200 µl (200 mg) / cm²
Diffusion cell	:	flow through system (1 cm²)
Receptor fluid	:	saline pH 3.0
Assay	:	HPLC
GLP	:	in compliance

Porcine ear obtained from the slaughter house immediately after slaughter and before steam cleaning were used for this experiment. The outer ear region was washed, carefully shaved and the skin was removed by dissection. Thickness of the dissected skin was approximately 400-500 µm. The skin was mounted in glass flow-through diffusion chamber with area open to diffusion of 1 cm². Each donor chamber was filled with 200µl (200 mg) of the test item and covered (occluded) with Parafilm™. Saline, pH 3.0, was pumped through the receptor chambers, with a flow rate of 1-2 ml/hour, and was collected in plastic vials which were replaced according to the sampling times and stored at – 20°C. The whole test system was set up in an incubator adjusted to 32 °C. After 30 min of contact the test item was removed from skin with shampoo solution. Following the washing procedure the donor chamber were filled with 1ml of saline pH 3.0 for monitoring skin integrity during the 24 hours of diffusion)-. The collecting vials were changed after 0.5, 1, 2, 4, 6, 8 and 24 hours and D&C orange 4 was analyzed.

At the end of the experiment the epidermal membrane was separated from the full thickness skin by heat. This technique as described by the applicant removes the horny layer and part of the upper stratum germinativum from the rest of the skin (lower stratum germinativum and upper dermis). After skin extraction the item bound in the tissues was quantified. Since the epidermis was separated from the dermis by heat separation method, the amount of dye found in the upper skin is considered by the applicant not to have passed the skin. The amount of penetrated test item found in the receptor solution plus that found in the lower skin extracts are considered as penetrated respectively absorbed.

Result
No measurable permeation through the skin occurred at any time point within the time frame of both experiments. The lowest detection limit under the conditions reported is 0.08 µg/ml in the first and 0.078 µg/ml in the second experiment. The maximal possible, calculated amount of the test item diffusiong across the skin barrier is 1.2 µg/cm^2 in the first and 1.1 µg/cm^2 in the second experiment. Together with the lower skin extract, the worst case consideration of penetrated test item is 2.4 µg/cm² (0.26 % of the applied dose) in the first and 4.2 µg/cm² (0.37 % of the applied dose) in the second experiment. It has to be emphasised that the values of the absportion without the lower skin are calculated and do not reflect real penetration. The mean recovery of the test item was 107.9 % in the first and 92.9 % in the second experiment.

Ref.: 23

Comment:

* The applied dose 200 mg/cm² is not relevant
* The use of a receptor phase at pH 3 is “non physiologic” and not documented,
* During all the diffusion period (24 hours) the stratum corneum is in contact with 1 ml of pH3 saline that is from the applicant “compatible” with the test product. No information is

provided on the effect of this permanent liquid in contact with the horny layer on the skin extraction or diffusion of the test compound.

* The heat separation is considered removing part of the stratum germinativum, in this case most of the epidermis is not taken into account for the estimation of the percutaneous absorption. No histological information is supporting this assertion. This is not acceptable to consider that stratum germinativum (the basal layer) is part of the "horny layer", i.e. a structure from which the tested compound will be exfoliated.
* The amount absorbed should be for both experiment (worst situation):
  - 1.2 µg (for the receptor fluid) + 2.4 µg (for the dermis) + 9.0 µg (for the epidermis) = 12.6 µg absorbed in 24 hours after a contact of 30 minutes
  - 1.1 µg (for the receptor fluid) + 3.1 µg (for the dermis) + 18.0 µg (for the epidermis) = 22.2 µg absorbed in 24 hours after a contact of 30 minutes
  - for the two studies associated, the mean total absorbed through the skin would be 17.4 µg in 24 hours after a contact of 30 minutes
* The study is not in accordance with the SCCNFP requirements.

Cutaneous absorption of D&C Orange 4, influence of carriers

Guideline	:	/
Test system	:	full back and flank pig skin (1000 µm), skin integrity by triated water
Contact time	:	30 minutes then washing with a shampoo, diffusion monitored during 24 hours
Test substance	:	D&C orange 4 in several formulations (at 0.5 % in a gel and at 0.13 % in a foam)
Purity	:	/
Batch no	:	/
Application	:	100 mg / cm²
Diffusion cell	:	flow through system
Receptor fluid	:	saline
Assay	:	HPLC
GLP	:	/

Split thickness pig skin samples from back and flank skin of a male (Schweizer Edelschwein) were used for this experiment, 1000 µm in thickness (stratum corneum, stratum germinativum and part of the dermis containing blood vessels). The surface of the skin which was in contact with the test substance during permeation-assay was 4 or 9 cm^2. The dermal absorption of Orange 4 was investigated from various formulations (gel and foam). 100 mg/cm² of the gel containing 0.5 % Orange 4 was applied to the skin (0.5 mg Orange 4 /cm^2) for 30 minutes and subsequently washed off with water and shampoo. 100 mg of the foam formulation containing 0.13 mg of Orange 4 (= 0.13 %) was applied to 9.1 cm^2 under similar conditions. In the flow-through system, the physiological receptor fluid in the acceptor chamber was constantly renewed (2.5-5 ml/h). The test item was sufficiently soluble in the receptor fluid (> 1mg/ml), thus not acting as a barrier to absorption. The receptor fluid was sampled after 16, 24 hours or in some experiments up to 88 hours. The content extracted from the skin (epidermis and upper dermis separated) after 24, 72 or 88 hours was determined in the same way.

Result

According to the applicant, the content of Orange 4 found in every single fraction of the receptor fluid was low despite the late depot often measured on the skin surface or in upper layers of the

stratum corneum. Taken together, these findings indicate that the part of Orange 4 which remains on or in the horny layer after the washing steps remains mainly on or in this layer, and that there is poor delivery into the receptor fluid over the observation period ranging from 24 to 88 hours in different experiments. Therefore the amounts found in the epidermis (skin surface) will not be considered for the calculation of the biologically available amount. In the case of the foam formulation, the worst case assumption considers a maximal amount of 12.9 ng/cm^2 as biologically available, while the same considerations for the other experiments lead to amounts of 557 ng/cm^2 respectively 1813 ng/cm^2 as biologically available. For risk assessment approach a worst case calculation of 1813 ng/cm^2 is justified.

Ref.: 27

Comment
This report is a compilation of two studies. The original reports with the complete experimental data are not in the documentation. Because of the lack of information this report is considered inadequate for the evaluation of the dermal absorption of D&C Orange 4.

2.8. Mutagenicity/Genotoxicity

2.8.1 Mutagenicity/Genotoxicity *in vitro*

Reverse Mutation Testing Using Bacteria

Guideline	:	OECD 471 (1997)
Species/Strain	:	*Salmonella typhimurium* TA1535, TA1537, TA98, TA100; *E. coli* wP2uvrA
Test item	:	D&C Orange 4
Batch n°	:	2097 AF
Lot n°	:	AJ3559
Purity	:	certified total colour content: 90%
Doses	:	33, 100, 333, 1000, 2500, 5000 µg/plate
Replicate	:	2 experiments (pre-incubation)
Metabolic Act.	:	uninduced Syrian Hamster liver homogenate
Positive controls	:	According to OECD Guideline
GLP	:	in compliance

Results
No increase of mutations induced by the test item in all strains and all conditions, was observed.

Conclusions
The test item is not mutagenic on bacterial cells.

Ref.: 20

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

Guideline	:	OECD 476 (1997)
Species/Strain	:	Mouse lymphoma L51878Y cells (forward mutation at thymidine kinase (TK+/-) locus
Test item	:	D&C Orange 4
Batch n°	:	2097 AF

Lot n° : AJ3559
Purity : certified total colour content: 90%
Doses : 6.9; 13.8; 27.5; 55.0; 110; 220 µg/plate (+/- S9) 1st experiment
28.1; 56.3; 112.5; 225; 450; 900 µg/plate (- S9) 2nd experiment
Positive controls : MMS (- S9); DMNA (+ S9)
Replicate : 2 experiments (2 cultures/each)
Metabolic Act. : uninduced Syrian Hamster liver homogenate
GLP : in compliance.

Results
Toxicity: in a preliminary study, a toxic effect was observed at 218.8 µg/ml (- S9) and at 109.4 µg/ml (+ S9) 4h treatment.
In a continuous treatment (-S9), a toxic effect was observed at 875.0 µg/ml, possibly due to protein binding of the test item.

Mutagenicity
Large and small colonies were counted in order to investigate the possible induction of chromosome aberrations.

Treatment	Culture 1		Culture 2		1st exp.	2nd exp.
	S.	L.	S.	L.	S.	L.
Pos. control MMS	157	22	210	123	639	146
DMNA 3-MC	138	28	114	121	-	-
Neg. control	24	11	32	50	80	31
	24	13	44	52	-	-

A reduction in the survival percentage of the cells was observed. No increase in mutation frequency under all conditions was observed.

Conclusion
In the test N-Nitrosodimethylamine (DMNA) is indicated with the sample employed. In the tables 3-MC (3-Methylcholanthrene) is indicated. Historical data are reported for both control. The test substance is not mutagenic in this assay.

Ref.: 21

2.8.2 Mutagenicity/Genotoxicity *in vivo*

***In vivo* Mammalian Erythrocyte Micronucleus Test**

The study report is not included.

Ref.: 22

2.9. Carcinogenicity

Skin painting, Mice

Skin painting studies in Swiss Webster mice were carried out with a series of 11 coal-tar-derived colors including Acid Orange 7. The treatment groups contained 50 males and 50 females and the control groups contained 100 males and 100 females. Mice were painted once weekly in an area that precluded oral exposure with 0.1 ml containing 1.0% Acid Orange 7 to a depilated 6 cm^2 area. Survival, body weight, and palpable growth were followed for a 18 month period. Microscopic examination which initially involved 50% of the treated animals was extended to include all tumours and grossly abnormal tissues and organs. There were no significant differences between treatment and control groups.

Ref.: 16

Other studies

Orange 4 is poorly absorbed from the intestine in mammals, but it is metabolized to sulphanilic acid and 1-amino-2-naphthol by micro-organisms in the gastro-intestinal tract which break down the azo linkage. Two papers describing the same study report that bladder implantation of pellets of 1-amino-2-naphthol hydrochloride in paraffin wax for 40 wk caused bladder cancer in mice. 1-Amino-2-naphthol hydrochloride showed no evidence of mutagenicity in *Salmonella typhimurium* bacteria with or without a rat liver activation system.

Ref.: a, b

Human studies

No data

2.10. Special investigations

No data

2.11. Safety evaluation

Not applicable

2.12. Conclusions

Physico-chemical properties
Absolute content of Acid Orange 7 has not been reported for any batch of the test material. The stability in a prototype formulation is not reported.

Toxicity
Acid Orange 7 is of very low acute toxicity. The NOAEL, derived from a 13-weeks oral toxicity study in rats, is 2.5 mg/kg/bw. Studies of effects on embryo-foetal development lead to a NOAEL of 5 mg/kg/bw for the maternal organism and 320 mg/kg/bw for the foetal organism.

Irritation and sensitization
The assessment of skin and eye irritation is not complying with the actual standards; nevertheless, there is no reason to ask for a repetition of these studies.
Acid Orange 7 is not a sensitiser.

Percutaneous absorption
- The use of a receptor phase at pH 3 is "non physiologic" and not documented,
- During all the diffusion period (24 hours) the stratum corneum is in contact with 1 ml of pH3 saline that is from the applicant "compatible" with the test product. No information is provided on the effect of this permanent liquid in contact with the horny layer on the skin extraction or diffusion of the test compound.
- The heat separation is considered removing part of the stratum germinativum, in this case most of the epidermis is not taken into account for the estimation of the percutaneous absorption. No histological information is supporting this assertion. This is not acceptable to consider that stratum germinativum (the basal layer) is part of the "horny layer", i.e. a structure from which the tested compound will be exfoliated.
- The amount absorbed should be for both experiment (worst case situation):
  * 1.2 µg (for the receptor fluid) + 2.4 µg (for the dermis) + 9.0 µg (for the epidermis) = 12.6 µg absorbed in 24 hours after a contact of 30 minutes
  * 1.1 µg (for the receptor fluid) + 3.1 µg (for the dermis) + 18.0 µg (for the epidermis) = 22.2 µg absorbed in 24 hours after a contact of 30 minutes
  * for the two studies associated, the mean total absorbed through the skin would be: 17.4 µg in 24 hours after a contact of 30 minutes
- The studies are not in accordance with the SCCNFP requirements.

Mutagenicity/genotoxicity
The test item has been tested for its potential mutagenicity/genotoxicity in two in vitro tests and in one in vivo test. The compound has been found unable to induce gene mutations in bacterial and mammalian cells treated in vitro, chromosome aberrations in mammalian cells treated in vitro.

Carcinogenicity
The sensitivity of the skin painting carcinogenicity test is low and it is unlikely that it would have identified a carcinogenic potential. An experiment where a metabolite of Orange 4 was implanted in the urinary bladder of mice is probably of little relevance to the use of the substance as a hair dye.

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 :

* percutaneous absorption study in accordance with the SCCNFP Notes of Guidance.

4. Other considerations

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5. Minority opinions

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