



Scientific Committee on Consumer Safety

SCCS

**OPINION ON
Acid Orange 7**

COLIPA n° C15



The SCCS adopted this opinion at its 10th plenary meeting
of 22 March 2011

Opinion on Acid Orange 7**About the Scientific Committees**

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

Scientific Committee members

Jürgen Angerer, Ulrike Bernauer, Claire Chambers, Qasim Chaudhry, Gisela Degen, Thomas Platzek, Suresh Chandra Rastogi, Vera Rogiers, Christophe Roussel, Tore Sanner, Jacqueline van Engelen, Maria Pilar Vinardell, Rosemary Waring, Ian R. White

Contact

European Commission
Health & Consumers
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-Sc6-Secretariat@ec.europa.eu

© European Union, 2011

ISSN 1831-4767

ISBN 978-92-79-30711-9

Doi:10.2772/96785

ND-AQ-11-004-EN-N

The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

ACKNOWLEDGMENTS

Prof. J. Angerer
Dr. C. Chambers
Prof. T. Platzek (chairman)
Dr. S.C. Rastogi (rapporteur)
Dr. C. Rousselle
Prof. T. Sanner
Dr. J. van Benthem (associate scientific adviser)
Prof. M.P. Vinardell
Dr. I.R. White

External experts

Dr. Mona-Lise Binderup National Food Institute, Denmark

Keywords: SCCS, scientific opinion, hair dye, Acid Orange 7, C15, CAS 633-96-5, EC 211-199-0, Directive 76/768/EEC

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on Acid Orange 7, 22 March 2011

This opinion has been subject to a commenting period of four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.

TABLE OF CONTENTS

ACKNOWLEDGMENTS	3
1. BACKGROUND	5
2. TERMS OF REFERENCE	5
3. OPINION	6
4. CONCLUSION	26
5. MINORITY OPINION	26
6. REFERENCES	27

Opinion on Acid Orange 7**1. BACKGROUND**

Submission I for this substance was submitted in September 2003 by COLIPA¹.

The Scientific Committee on Cosmetic and Non-Food Products (SCCNFP) adopted on 23 April 2004 its opinion (SCCNFP/0785/04) on Acid Orange 7. In the conclusions it was said:

- *data are missing about the content of the substance in the batch testing material, and the stability of the substance was not reported.*
- *before assessment of the substance a percutaneous absorption study is needed in accordance with the SCCNFP Notes of Guidance.*

Acid Orange 7 is identical with CI 15510 also used as a colouring agent "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."

Submission II of Acid Orange 7 was submitted by COLIPA in July 2005. According to this submission acid orange 7 is a non-reactive dye, used as direct hair colouring agent up to an on-head concentration of 0.5% in non-oxidative as well as in oxidative hair dye formulations. For non-oxidative dye formulations it is common practice to apply 35-50 mL of undiluted formulations, while for oxidative colouring formulations 100 mL are applied after mixing with hydrogen peroxide. The application time for both formulations covers a period of 30 minutes followed by washing off with water and shampoo. It is assumed that application may be repeated at weekly intervals for non-oxidative and monthly intervals for oxidative dyes.

Submission II presents updated scientific data on the above mentioned substance in line with the second step of the strategy for the evaluation of hair dyes (<http://europa.eu.int/comm/enterprise/cosmetics/doc/hairdyestrategyinternet.pdf>) within the framework of the Cosmetics Directive 76/768/EEC.

2. TERMS OF REFERENCE

1. *Does the Scientific Committee on Consumer Safety (SCCS) consider Acid Orange 7 safe for use in non-oxidative hair dye formulations with a concentration on-head of 0.5% taken into account the scientific data provided?*
2. *Does the SCCS consider Acid Orange 7 safe for use in oxidative hair dye formulations with a concentration on-head of 0.5% taken into account the scientific data provided?*
3. *Does the SCCS recommend any restrictions with regard to the use of Acid Orange 7 in oxidative or non-oxidative hair dye formulations (e.g. max conc. in the finish cosmetic product, dilution ratio with hydrogen peroxide, warning?)*

¹ COLIPA - European Cosmetics Toiletry and Perfumery Association

Opinion on Acid Orange 7**3. OPINION****3.1. Chemical and Physical Specifications****3.1.1. Chemical identity****3.1.1.1. Primary name and/or INCI name**

Acid Orange 7 (INCI)

3.1.1.2. Chemical names

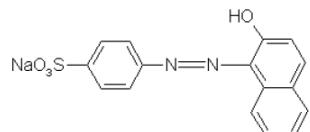
Sodium 4-[(2-hydroxy-1-naphthyl)azo]benzene sulfonate
4-[(2-hydroxy-1-naphthyl)azo]benzenesulfonic acid, monosodium salt,

3.1.1.3 Trade names and abbreviations

Orange 205
Acid Orange 7 monosodium salt
Orange II
D&C Orange 4
CI 15510
COLIPA C015

3.1.1.4 CAS / EC number

CAS: 633-96-5
EC: 211-199-0

3.1.1.5 Structural formula**3.1.1.6 Empirical formula**

Formula: C₁₆H₁₁N₂NaSO₄

3.1.2 Physical form

Orange powder, odourless

3.1.3 Molecular weight

Molecular weight: 350.3 g/mol

Opinion on Acid Orange 7**3.1.4 Purity, composition and substance codes**

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.

Purity: 90% (total colour content)

Relative chromatographic purity

(HPLC - UV/VIS peak area method): 99-100% at 210 nm, 254 nm, 480 nm)

Batch Comparison

Lot n°	R0073770	AJ3559	AK5453	AL1478
Batch	0201212137	2097 AF		DC04/7
		FDA certified	FDA certified	FDA certified
Total colour	99% (HPLC)	90%	91%	96%
Volatile matter		6.7%		2.7%
NaCl		2.7%		1.2%
Na₂SO₄		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	/		/
Lead		/		/
Arsenic		/		/

Comment

- Purity and impurities in all batches is reported in a table (described above), but analytical reports were not submitted.
- The content of Acid Orange 7 in the test materials has not been measured using a standard reference material. The colour content (measured as HPLC peak area?) of various batches, reported in the table above, may not represent Acid Orange 7 content.
- Colour content in the batch AK5453 is only 91%, but impurities in this batch are not reported.

3.1.5 Impurities / accompanying contaminants

See 3.1.4

3.1.6 Solubility

Water: 11% at 30°C

Saline: 2%

DMSO: 10%

Formulation: 2%

Comment

Method for the determination of water solubility was not reported

3.1.7 Partition coefficient (Log P_{ow})

Log P_{ow}: 1.4 (EC Method A.8)

3.1.8 Additional physicochemical specifications

Melting point: 164 °C (decomposition)

Boiling point: /

Opinion on Acid Orange 7

Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
UV/Visible spectrum:	/λ max at 229 nm and 485 nm

3.1.9. Stability

Aqueous solutions were stable ≥72 hours.

Solutions/suspensions of Acid Orange 7 (0.025 mg/ml – 32 mg/ml) in 1% aqueous carboxymethylcellulose, used in 13 week chronic toxicity and developmental toxicity studies, were stable for 11 days storage period.

General Comments on Physico-chemical characterisation

- The content of Acid Orange 7 in the test materials has not been measured using a standard reference material. The colour content (measured as HPLC peak area?) of various batches may not represent Acid Orange 7 content
- Colour content in the batch AK5453 is only 91%, but impurities in this batch are not reported.
- Stability of Acid Orange 7 in typical hair dye formulations is not reported.
- The stability in an oxidative environment has not been demonstrated

3.2. Function and uses

Acid Orange 7, a non reactive dye, is used as a direct hair colouring agent up to an on-head concentration of 0.5% in non-oxidative as well as in oxidative hair dye formulations.

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.

Opinion on Acid Orange 7**3.3. Toxicological Evaluation**

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

Moreover, the applicant of the dossier carried out a literature search using the ChemI Plussystem, a search system including 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.

3.3.1. Acute toxicity**3.3.1.1. Acute oral toxicity**

LD50 (rats) > 10,000 mg/kg bw
 LD50 (♀ mice) > 10,000 mg/kg bw

Ref.: 1

LD50 (♀ +♂ rats) > 10,000 mg/kg bw.

Ref.: 2, 3, 4

LD50 (♀ +♂ 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.

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity**3.3.2.1. Skin irritation**

Guideline: /
 Species/strain: albino rabbits (1.5 – 2.0 kg)
 Size: 4 males
 Test item: Orange II
 Batch: /
 Purity: /
 Dose: 100 mg
 GLP: /

Opinion on Acid Orange 7

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

Result

Orange II 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 a 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.

3.3.2.2. Mucous membrane irritation

Primary eye irritation, undiluted test compound

Guideline:	/
Species/strain:	rabbit
Group size:	3 males
Test substance:	Orange II
Batch:	/
Purity:	/
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.
Batch:	/
Purity:	/
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.

Opinion on Acid Orange 7**3.3.3. Skin 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
Batch:	/
Purity:	> 85%
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 after 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 considered as a 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:	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)
Positive control:	p-phenylenediamine (PPD) 1% /w/v) in DMSO
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 [³H]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 [³H]-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 3HTdR at least 3-fold or greater than that recorded in control mice, as indicated by the stimulation index (S.I.).

Opinion on Acid Orange 7**Results**

Treatment	Concentration	Stimulation index
Test item in DMSO	0.3%	1.0
	1%	1.5
	3%	2.1
	9%	1.2
Test item in acetone/water mixed with olive oil	0.3%	1.3
	1%	1.4
	3%	1.4
	9%	1.0
PPD in DMSO	1%	7.9

Conclusion

Based on the result in this LLNA in mice, D&C Orange 4 is not a skin sensitizer under defined experimental conditions in the two vehicles tested (DMSO or acetone/water mixed with olive oil).

Ref.: 11

Human studies

Two human studies were performed.

Firstly, a patch test among 10 volunteers (5 were tested with the individual dye and other 5 with the hair dye formulations). The hair dye formulation consisted on the hair dye in a base formulation containing water, propylene carbonate, alcohol denat, lactic acid, PVMA/MA decadiene crosspolymer, dimethicone copolyol, fragrance, sodium hydroxide. The formulation was tested at 1, 3, and 10% in petrolatum. The individual hair dye was tested at 0.2, 0.6 and 2% in petrolatum.

In the second study, 40 hairdressers with a known allergy to PPD (p-phenylenediamine) and/or DTS (toluene-2,5-diamine) and/or ONPPD (ortho-nitro-p-phenylenediamine) were selected for the cross-reaction study. They were tested with the highest non-irritant concentration of the single hair dye and with the highest non-irritant concentration of the formulation.

Exclusion criteria were applied in both studies based on the use of antihistamines or systemic corticosteroids, moderate to severe skin exposure to the sun or to artificial UV light 1 week before investigation, severe systemic allergic reactions in the history or dermatitis on the skin of the back or severe dermatitis on other parts of the body.

Results

In the first study no irritant reaction to any of the concentrations were observed, either to the individual hair dyes or to the hair dye formulations. Based on these results, the highest concentration was used to perform the study for phase 2.

No positive reactions to the hair dye and to the hair dye formulation were observed in the second study with the hairdressers.

Ref.: 33

Comment

These studies show that cross-sensitivity does not occur in individuals with known contact allergy to the main indicators for hair dye contact allergy.

3.3.4. Dermal / percutaneous absorption

Guideline: OECD 428 (1999)

Opinion on Acid Orange 7

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)
Batch:	2097AF (raw material)
Purity:	90% (raw material)
Application:	200 µl (200 mg) / cm ²
Diffusion cell:	flow through system (1 cm ²)
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 200 µl (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 diffusing across the skin barrier is 1.2 µg/cm² in the first and 1.1 µg/cm² 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 absorption 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 of 200 mg/cm² is too high
- * The use of a receptor phase at pH 3 is "non physiologic" and not justified
- * During all the diffusion period (24 hours) the stratum corneum is in contact with 1 ml of pH 3 saline that is according to the applicant "compatible" with the test product. No

Opinion on Acid Orange 7

- 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 histology was performed. It is not acceptable to consider that stratum germinativum (the basal layer) as part of the "horny layer", i.e. a structure from which the tested compound will be exfoliated.
 - * The amount absorbed should be for both experiments (worst situation):
 - $1.2 \mu\text{g}$ (for the receptor fluid) + $2.4 \mu\text{g}$ (for the dermis) + $9.0 \mu\text{g}$ (for the epidermis) = $12.6 \mu\text{g}$ absorbed in 24 hours after a contact of 30 minutes
 - $1.1 \mu\text{g}$ (for the receptor fluid) + $3.1 \mu\text{g}$ (for the dermis) + $18.0 \mu\text{g}$ (for the epidermis) = $22.2 \mu\text{g}$ absorbed in 24 hours after a contact of 30 minutes
 - for the two studies associated, the mean total absorbed through the skin would be $17.4 \mu\text{g}$ in 24 hours after a contact of 30 minutes
 - * The study is not in accordance with the SCCS requirements.

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

Guideline:	/
Test system:	full back and flank pig skin ($1000 \mu\text{m}$), 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)
Batch:	/
Purity:	/
Application:	100 mg/cm^2
Diffusion cell:	flow through system
Receptor fluid:	saline
Assay:	HPLC
GLP:	/

Split thickness pig skin samples from back and flank skin of a male animal (Schweizer Edelschwein) were used for this experiment, $1000 \mu\text{m}$ 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/cm^2 of the gel containing 0.5% Orange 4 was applied to the skin ($0.5 \text{ mg Orange 4 /cm}^2$) 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 ($2.5\text{-}5 \text{ ml/h}$). The test item was sufficiently soluble in the receptor fluid ($> 1\text{mg/ml}$), 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

Opinion on Acid Orange 7

assumption considers a maximal amount of 12.9 ng/cm² as biologically available, while the same considerations for the other experiments lead to amounts of 557 ng/cm² respectively 1813 ng/cm² as biologically available. For risk assessment approach a worst case assumption of 1813 ng/cm² 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.

New study, submission 2005

Guideline:	OECD Guideline 428
Tissue:	fresh dermatomed pig ear skin samples 300 µm thickness
Group size:	17 (1 was excluded) 3 experiment x 6 cells
Skin integrity:	conductivity < 900 µS
Diffusion cell:	glass flow-through diffusion cells
Test substance:	D&C Orange 4
Batch:	T1 E 2000064 4
Purity:	90%
Test item:	SC Clear + 0.5% D&C Orange 4 (Lot AJ 3559)
Dose:	20 µL per cm ²
Dose of test substance:	100 µg/cm ²
Receptor fluid:	Saline (0.9% NaCl)
Solubility receptor fluid:	/
Stability receptor fluid:	≥ 72 hours in water
Method of Analysis:	HPLC analysis and UV/VIS
GLP:	in compliance
Study date:	July 2005

The relevant component of SC Clear + 0.5% D&C Orange 4 was assessed for its potential for dermal absorption on porcine skin.

Three independent experiments were performed with 6 diffusion cells per experiment, of which, due to skin integrity problems, one cell could not be included in the calculations.

Thus, a total of 17 cells were used for the quantification of dermal absorption.

The study was performed on fresh dermatomed pig skin samples mounted on diffusion cells between donor and receptor chambers. The conductivity across the skin samples of each cell was determined before treatment and after the last sampling as a measure of skin integrity. 20 µL of the formulation was applied to each skin sample for 30 minutes.

Results

Chamber	Experiment I					
	1	2	3	4	5	6
Dermal absorption µg/cm ²	1.63	1.23	4.61	1.57	3.05	0.943
Dermal absorption %	1.84	1.33	4.87	1.74	3.25	1.07
Experiment II						
Chamber	1	2	3	4	5	6
Dermal absorption µg/cm ²	0.359	0.213	0.662	0.285	0.754	0.872
Dermal absorption %	0.43	0.25	0.80	0.33	0.89	1.01
Experiment III						
Chamber	1	2	3*	4	5	6
Dermal absorption µg/cm ²	0.927	0.367	2.77	0.081	1.12	0.533
Dermal absorption %	1.02	0.42	3.21	0.09	1.27	0.6
Mean dermal absorption Experiments I-III ± SD	$1.13 \pm 1.15 \mu\text{g}/\text{cm}^2$					
	$1.25 \pm 1.25\%$					

Opinion on Acid Orange 7

* value not used for the calculation of the mean dermal absorption, since the integrity of the skin sample was not given

Under the reported conditions, the dermal absorption of D&C Orange 4 is $1.13 \pm 1.15 \mu\text{g}/\text{cm}^2$ or $1.25 \pm 1.20\%$ (mean value of 17 diffusion cells (17 donors)).

Ref.: 31

Comments

There is a high variability among the different cells ranging from 0.081 to $4.61 \mu\text{g}/\text{cm}^2$ (0.09 to 4.87%). Therefore, the mean value + 2SD ($3.43 \mu\text{g}/\text{cm}^2$ ($1.13 + 2 \times 1.15$)) will be used to calculate MOS.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (14 days) oral / dermal / inhalation 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:	2097AF, Lot No. AJ 3559
Purity:	90%
Dosages:	0, 10, 60, 100, 300, 1000 mg/kg bw/day by oral gavage
GLP:	/

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

The test material (purity 90%, Batch 2097AF; Lot no. AJ3559) was homogenized in bi-distilled water containing 1% CMC.

The animals (5 males, 5 females, each concentration) were treated by oral gavage, once daily, 7 days per week for 14 days at doses of 0, 10, 60, 100, 300, 1000 mg/kg bw.

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

All animals survived until scheduled necropsy. Discoloration of faeces resulting from the oral administration of Acid Orange 7 was observed. No changes were observed in the body and body weight gains of the rats, nor in the food consumption. A significant increase in the absolute and relative spleen weight was observed in the rats treated at the doses of 60, 100, 300 and 1000 mg/kg bw/day. At 10 mg/kg bw/day this increased was also observed but was not statistically significant. Enlarged spleens were macroscopically observed in all rats treated at the dose of 100, 300 and 1000 mg/kg bw/day. Extramedullary hemopoiesis was observed in all rats treated at the dose of 10 and 60 mg/kg bw/day (no histological examination was performed on other groups). This was considered related to the tested compound.

Conclusion

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 dose levels of 0, 2.5, 5, 10 mg/kg bw/day was envisaged.

Ref.: 12

Opinion on Acid Orange 7**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/day; by oral gavage
 GLP: /

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

The test material (purity 90%, Batch 2097AF; Lot no AJ3559) was homogenized in bi-distilled water containing 1% CMC.

The animals (5 males, 5 females, each concentration) were treated by oral gavage, once daily, 7 days per week for 14 days at doses of 0, 2.5, 5, 10 mg/kg bw.

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

All animals survived until scheduled necropsy and no clinical signs were observed during the study. No changes were observed in the body and body weight gains of the rats, nor in the food consumption. Methemoglobin levels were significantly increased in the female treated at the dose of 10 mg/kg bw/day after 14 days treatment. This increase was also observed in males at the same dose but was not statistically significant. Heinz bodies were not reported in these animals. Bilirubin levels were also increased in the male rats treated at 10 mg/kg bw/day but this increase was not considered related to the tested compound. No test compound related findings in organ weight were observed. No microscopic findings related to Acid Orange 7 were reported.

Conclusion

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/day.

Ref.: 13

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation 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/day; by oral gavage
 GLP: in compliance

Opinion on Acid Orange 7

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 g (mean 141 g) for males and between 98-143 g (mean 123 g) for females.

The test material 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

All rats survived during the study. No clinical signs were observed. No test-related differences in grip strength were noted in all treated animals, when compared with the controls. A dose related decrease in locomotor activity was observed in male treated at the dose of 5 and 10 mg/kg bw/day and in female at the dose of 10 mg/kg bw/day.

Changes in haematology were evident in males treated with 5 and 10 mg/kg bw/day. Changes in haematological parameters like increased methemoglobin levels in males up to 2.5 mg/kg bw/day and females up to 5 mg/kg bw/day, decreased haemoglobin levels in males (5 and 10 mg/kg bw/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 reticulocyte counts in males treated with 2.5 mg/kg bw/day were within the upper levels of the historical control data.

When compared with similarly high values at 5 mg/kg bw/day, there was no correlation to pathomorphological (extramedullary hemopoiesis in the spleen) or other haematological parameters at 2.5 mg/kg bw/day.

In males treated with 10 mg/kg bw/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 bw/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 Observed Adverse Effect Level (NOAEL) of Acid Orange 7 was considered to be in the general range around 2.5 mg/kg bw/day. This dose led to borderline effects on met-hemoglobin and reticulocytes counts without concurrent effects on the spleen. The NOAEL for females was set to 2.5 mg/kg bw/ day by the study authors.

Ref.: 14

Comment

It should be noted that at this dose, some slight early haematological effects (increased met-haemoglobin levels in males and increased reticulocytes count) are already observed. Even if there are in the upper limits of the historical control values, this finding could be test article related. Therefore, the dose of 2.5 mg/kg bw /day is considered as the LOAEL.

90-day 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

Opinion on Acid Orange 7

There was no mortality or any evidence of systemic toxicity. Haematological values, growth responses and urinary components were normal. Gross autopsies disclosed no dose-related findings.

Ref.: 15

3.3.5.3. Chronic (> 12 months) 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 cm².

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 3.3.7. Carcinogenicity.

3.3.6. Mutagenicity / Genotoxicity

3.3.6.1. Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline:	OECD 471 (1997)
Species/Strain:	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537 and <i>Escherichia coli</i> WP2uvrA
Replicates:	triplicates in 2 individual experiments
Test substance:	D&C Orange 4
Solvent:	DMSO
Batch nr:	2097AF
Purity:	90% (certified total colour content)
Concentration:	0, 33, 100, 333, 1000, 2500 and 5000 µg/plate without and with S9-mix
Treatment:	pre-incubation method with 30 minutes pre-incubation and at least 48 h incubation
GLP:	in compliance
Study date:	27 May 1999 – 7 September 1999

D&C Orange 4 was investigated for the induction of gene mutations in strains of *Salmonella typhimurium* and *Escherichia coli* (Ames test). Liver S9-fraction from untreated 7-8 weeks old male Syrian hamsters was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-experiment for toxicity and mutation induction with all strains both without and with S9-mix. Toxicity was evaluated for 8

Opinion on Acid Orange 7

concentrations up to the prescribed maximum concentration of 5000 µg/plate on the basis of a reduction in the number of revertant colonies and/or clearing of the bacterial background lawn. Because relevant toxic effects were only observed in one of the strains at the maximal concentration, 5000 µg/plate was used as the top concentration. Since in this pre-experiment evaluable plates were obtained for five concentrations or more in all strains used, the pre-experiment is reported as experiment I. Both experiments were performed with the pre-incubation method. Negative and positive controls were in accordance with the OECD guideline.

Results

No precipitation occurred up to the highest concentration tested. Toxic effects evident as clearing of the bacterial background lawn were observed in experiment I in TA98 at the highest dose (5000 µg/plate) and in experiment II in TA98 at 2500 and 5000 µg/plate and in TA1535 at 1000 and 2500 µg/plate for both strains exclusively in the presence of S9-mix. A biologically relevant increase in revertant colonies was not observed in any of the strains tested at any dose level in the absence or presence of S9-mix in both experiments. An isolated increase in strain TA98 exceeding the threshold of twice the number of revertants of the corresponding solvent control was observed in the first experiment at 333 µg/plate in the presence of S9-mix. Since this increase was not reproduced in the second experiment under identical experimental conditions controls, it was considered not biologically relevant.

Conclusion

Under the experimental conditions used D&C Orange 4 was not mutagenic in this gene mutation tests in bacteria both in the absence and the presence of S9 metabolic activation.

Ref.: 20

In Vitro Mammalian Cell Gene Mutation Test

Guideline:	OECD 476 (1997)
Species/Strain:	L5178Y Mouse lymphoma cells
Replicates:	duplicates in 2 independent experiments
Test substance:	D&C Orange 4
Solvent:	deionised water
Batch no.:	2097 AF
Purity:	90% (certified total colour content)
Concentrations:	Experiment I: 0, 13.8, 27.5, 55, 110 and 220 µg/ml without and with S9-mix Experiment II: 0, 28.1, 56.3, 112.5, 225, 450 and 900 µg/plate without S9-mix
Treatment	Experiment I: 4 h treatment without and with S9-mix; expression period 72 h and selection period of 10-15 days Experiment II: 24 h treatment without S9-mix; expression period 72 h and selection period of 10-15 days
GLP:	in compliance.
Study date:	25 May 1999 – 13 December 1999

D&C Orange 4 was assayed for gene mutations at the *tk* locus of mouse lymphoma cells both in the absence and presence of S9 metabolic activation. Liver S9-fraction from untreated 7-8 weeks old male Syrian hamsters was used as exogenous metabolic activation system. Test concentrations were based on the results of a pre-test on toxicity measuring relative suspension growth and relative total growth. In the main tests, cells were treated for 4 h (experiment I) or 24 h (experiment II) followed by an expression period of 72 h to fix the DNA damage into a stable *tk* mutation. Toxicity was measured in the main experiments as percentage relative total growth of the treated cultures relative to the total growth of the solvent control cultures. To discriminate between large (indicative for

Opinion on Acid Orange 7

mutagenic effects) and small colonies (indicative for a clastogenic effect) colony seizing was performed. Negative and positive controls were in accordance with the OECD guideline.

Results

The recommended toxic range of approximately 10-20% survival compared to the concurrent negative controls was never covered in any of the experiments.

A dose dependent and biologically relevant increase in the mutant frequency was not observed up to the maximal concentrations tested. The ratio of small *versus* large colonies was not shifted as compared to the solvent control.

Conclusion

Under the experimental conditions used, D&C Orange 4 was not mutagenic in this mouse lymphoma assay at the *tk* locus.

Ref.: 21

Comment

The appropriate level of toxicity (reduction of the relative total growth to 10-20%) was not reached pointing to insufficient exposure of the cells. However, higher concentrations, separated with a factor 2, in an additional experiment resulted in no surviving cells.

3.3.6.2 Mutagenicity/Genotoxicity *in vivo*

In vivo Mammalian Erythrocyte Micronucleus Test

Guideline:	OECD 474 (1997)
Species/strain:	NMRI mice
Group size:	5 mice/sex/group
Test substance:	D&C Orange 4
Batch no:	DC04/7
Purity:	96% (certified total colour content)
Dose level:	0, 500, 1000 and 2000 mg/kg bw
Route:	oral gavage
Vehicle:	deionised water
Sacrifice times:	24 h after treatment for all concentrations, 48 h for the high dose only.
GLP:	in compliance
Study date:	23 July 2002 – 19 August 2003

D&C Orange 4 has been investigated for the induction of micronuclei in bone marrow cells of mice. Test doses were based on the results of a pre-experiment with 2000 mg/kg bw on acute toxicity at various intervals of 1, 2-4, 6, 24, 30 and 48 h after start of treatment. In the main experiment mice were exposed, after starvation for 18 h, orally to 0, 500, 1000 and 2000 mg/kg bw. Bone marrow cells were collected 24 h or 48 h (high dose only) after dosing. The mice of the high dose group were examined for acute toxic symptoms at intervals of around 1, 2-4, 6 and 24 h after treatment. Toxicity and thus exposure of the target cells was determined by measuring the ratio between polychromatic and normochromatric erythrocytes (PCE/NCE). Bone marrow preparations were stained with May-Grünwald/Giemsa and examined microscopically for the PCE/NCE ratio and micronuclei. Additional animals (2 mice/dose/sex) were dosed, after starvation for 18 h, with the highest dose and the vehicle for blood sampling. Blood was sampled 1 and 4 h after treatment to analytically demonstrate the bioavailability of D&C Orange 4. Negative and positive controls were in accordance with the OECD guideline.

Results

In the pre-experiment on acute toxicity with an exposure of 2000 mg/kg bw D&C Orange 4 exclusively a ruffled fur was found up to 4 h after administration. In the main experiment reduction of spontaneous activity, eyelid closure and ruffled fur were found at 2000 mg/kg bw up to 6 or 24 h (ruffled fur only).

Opinion on Acid Orange 7

Treatment with D&C Orange 4 did not result in a decreased PCE/NCE ratios compared to the untreated controls indicating that D&C Orange 4 had no cytotoxic properties in the bone marrow. However, the quantitative analysis of the test item in the plasma of the treated animals showed significant amounts of D&C Orange 4 after 1 h. This level dropped after 4 h. Biologically relevant or statistically significant increases in the number of micronucleated PCEs compared to the concurrent vehicle controls were not found at any dose tested, neither 24 nor 48 h after treatment and neither for males nor for females.

Conclusion

Under the experimental conditions used D&C Orange 4 did not induce a biologically relevant increase in the number of PCEs with micronuclei in bone marrow cells of treated mice and, consequently, D&C Orange 4 is not genotoxic (clastogenic and/or aneugenic) in bone marrow cells of mice.

Ref.: 22

3.3.7. Carcinogenicity**Skin painting, Mice**

Skin painting studies in Swiss Webster mice were carried out with a series of 11 coal-tar-derived colours 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 cm² 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 sulphuric 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 weeks 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.: 29, 30

Human studies

No data submitted

3.3.8. Reproductive toxicity**3.3.8.1. Two generation reproduction toxicity**

No data submitted

3.3.8.2. Teratogenicity**Dose Range Finding Study for effects on Embryo-Foetal Development**

Guideline: /

Opinion on Acid Orange 7

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/day; 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 g at beginning of the study.

Test material (purity 90%; Batch 2097AF, Lot 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/day.

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

Maternal toxicity: Two females from the 1000 mg/kg bw/day group died after four or five administrations. Prior to death, the females felt cold and showed general poor conditions. Reduced food consumption and body weight gain, orange-brown discoloured urine and faeces, ruffled fur and prostrations were also observed in this tested group. At 300 mg/kg bw/day, a slight decrease in food consumption and body weight gain was also observed in the first half of the treatment period. Discoloration of urine and faeces were also observed at the dose of 300 and 100 mg/kg bw/day. In the females from all tested groups, the spleen appeared enlarged and significant dose-related increased spleen weights were observed in all treated rats.

Foetal toxicity: No abnormalities were observed in any foetuses. In the foetuses of group treated at 1000 mg/kg bw/day, a slight decrease in the mean body weight was observed.

Conclusion

With the exception of a slight reduced mean foetal body weight at 1000 mg/kg bw/day, 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/day; by oral gavage (day 6 to 17 p.c.)
GLP:	in compliance

Opinion on Acid Orange 7

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 g.

The test material 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 (Ref. 13, 18) 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

Maternal toxicity: All females survived and no clinical signs were noted in the treated rats. Reduced food consumption and body weight gain, orange-brown discoloured urine and faeces and ruffled fur were observed in the high dose group. At 40 mg/kg bw/day, a slight decrease in food consumption was also observed. Total resorptions were observed in 2 females in the high dose group. In the females treated at the dose of 40 and 320 mg/kg bw/day, a significant dose-related increased spleen weights was observed.

Foetal toxicity: No abnormalities related to the treatment were observed in any foetuses. In the foetuses of group treated at 1000 mg/kg bw/day (dose not used), a slight decrease in the mean body weight was observed.

Conclusion

Based on the result, the No-Observable-Adverse-Effect-Level (NOAEL) of Acid Orange 7 was considered to be 5 mg/kg bw/day for the maternal organism and 320 mg/kg bw/day for the foetal organism.

Ref.: 19

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

3.3.10.1. Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

Opinion on Acid Orange 7**3.3.13. Safety evaluation (including calculation of the MoS)****CALCULATION OF THE MARGIN OF SAFETY****Acid Orange 7
(non-oxidative conditions)**

Absorption through the skin	A (mean + 2SD)	= 3.43 µg/cm²
Skin Area surface	SAS	= 580 cm²
Dermal absorption per treatment	SAS x A x 0.001	= 1.99 mg
Typical body weight of human		= 60 kg
Systemic exposure dose (SED)	SAS x A x 0.001/60	= 0.033 mg/kg bw
Lowest Observed Adverse Effect Level (13-week, oral, rat)	LOAEL	= 2.5 mg/kg bw/d
Adjusted by factor 3		= 0.83 mg/kg bw/d

MOS	NOAEL / SED	= 25

3.3.14. Discussion*Physico-chemical properties*

Acid Orange 7 is intended for use in oxidative and non-oxidative hair dye formulations as a direct dye at a maximum concentration of 0.5%. Acid Orange 7 is also permitted to be used as a cosmetic colorant, listed as CI 15510 in Annex IV of the EU Cosmetic Directive.

The content of Acid Orange 7 in the test materials has not been measured using a standard reference material. The colour content (measured as HPLC peak area?) of various batches, reported in the table above, may not represent Acid Orange 7 content. Purity and impurities of all batches is not documented.

Colour content in one of the batches is only 91%, but impurities in this batch are not reported. The stability in of Acid Orange 7 in typical hair dye formulations is not reported. The stability in an oxidative environment has not been demonstrated.

Toxicity

Acid Orange 7 is of low acute toxicity.

The NOAEL of a 13-weeks oral toxicity study in rats, was set to 2.5 mg/kg bw/d by the study authors. It should be noted that at this dose, some slight early haematological effects (increased met-haemoglobin levels in males and increased reticulocytes count) are already observed. Even if they are in the upper limits of the historical control values, this finding could be test article related. Therefore the dose of 2.5 mg/kg bw/d should be considered as a LOAEL.

Studies of effects on embryo-foetal development lead to a NOAEL of 5 mg/kg bw/d for maternal toxicity and 320 mg/kg bw/d for foetal toxicity.

Irritation and sensitization

The assessment of skin and eye irritation does not comply with the current standards; nevertheless, there is no reason to ask for a repetition of these studies.

According to the studies available, Acid Orange 7 is not irritating to rabbit skin and not a sensitisier.

Percutaneous absorption

Under the reported conditions, the dermal absorption of Acid Orange 7 is $1.13 \pm 1.15 \mu\text{g}/\text{cm}^2$ or $1.25 \pm 1.20\%$ (mean value of 17 diffusion cells). There is a high variability

Opinion on Acid Orange 7

among the different cells ranging from 0.081 to 4.61 µg/cm² (0.09 to 4.87%). The mean value + 2SD of 3.43 µg/cm² (1.13 + 2 x 1.15) will be used to calculate MOS.

Mutagenicity/genotoxicity

Overall, the genotoxicity of Acid Orange 7 is investigated in valid genotoxicity tests for the 3 endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Gene mutations were investigated in *in vitro* test whereas chromosome aberrations and aneuploidy were tested in an *in vivo* test only. Acid Orange 7 was neither mutagenic in an gene mutation test in bacteria nor in an *in vitro* gene mutation test in mammalian cells. Acid Orange 7 did not induce an increase in bone marrow cells with micronuclei in an *in vivo* micronucleus test in mice. Consequently, Acid Orange 7 can be considered to have no *in vivo* genotoxic potential and additional tests are unnecessary.

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.

4. CONCLUSION

Based on the low margin of safety, the SCCS is of the opinion that the use of Acid Orange 7 as a hair dye ingredient up to a final on-head concentration of 0.5% under oxidative and non-oxidative conditions poses a risk to the health of the consumer.

No dermal absorption study was performed under oxidative conditions. No data on the stability in an oxidative environment has been provided.

The safety of the use of Acid Orange 7 (CI15510) as a cosmetic colorant should be assessed.

5. MINORITY OPINION

Not applicable

6. REFERENCES

Submission I, 2003

1. Dipali Roy & J. Saha; Acute Toxicity of dyes used in drugs and cosmetics; The Eastern Pharmacist; May 1981
2. C.S. Weil; Biometrics 8: 249-263, 1952
3. E.C. Hagen; Assoc of Food and Drug Officials of US, 1969
4. R.L. Singh; S.K. Khanna; Acute and short tem toxicity studies on Orange II; Vet. & Human Toxicol. 29, 300-304, 1987
5. The acute oral toxicity of D&C Orange 4; Report 81998; December 1961; Color Master file entry no.45.
6. J.H. Draize; W. Wood, G. Calvery; J. Pharmacol. Exp. Therap., 82 (1944), 377
7. D. Roy, J. Saha; Acute Toxicity of dyes used in drugs and cosmetics; The Eastern Pharmacist-May 1981
8. C.M. Burnett & D.L. Opdyke 1971. CTFA Cosmetics J, 3, 18 cited in Bibra Toxicological Profile
9. /
10. R.L. Guest, Magnusson & Kligman Maximization Study in the Guinea Pig; Safepharm Project No. 140/80R; Test Report, Derby; DE1 2BT; U.K; 1989
11. M.Christ, MDS Pharma Services; Saint Germain sur l'Arbresle; France; Study no 762/030
12. E. Rosner, 14-day oral toxicity (gavage) study in the rat ; RCC Report No. 736288; Itingen CH, October 1999
13. E. Rosner, 14-day oral toxicity (gavage) study in the rat ; RCC Report No. 746460; Itingen CH, October 15, 1999
14. H.J. Hamann, D. Richard, N. Robert, C. Knuppe, 13-week oral toxicity (gavage) study in rat with D&C Orange 4 (C.I. 15510), RCC Project No. 736312, Test Report , Itingen /CH; 2000
15. Color Additive Master File No. 9; Entry 54; Submitted to the Toilet Goods Association; 1962 (sample 82574)
16. Steven Carson. Skin painting study in mice on 11 FD&C and D&C Colors;; J. Toxicol.- Cut & Ocular Toxicol. 3(3), 309-331 (1984)
17. Report, skin painting studies in mice; Food and Drug Research Laboratories; submitted to Toilet Goods Association; December 31, 1963
18. H. Becker, K. Biedermann, Dose range finding sudy for the effects on embryo fetal development in the rat; RCC-Report No. 733950; Itingen/CH, July, 27 1999
19. H. Becker, K. Biedermann, study for effects on embryo-fetal development in the rat with D&C Orange 4 (C.I. 15510), RCC Project No. 733961, Test Report, CH-Itingen; 27.4.2000
20. H.E. Wollny; Salmonella typhimurium and Escherichia coli reverse mutation assay for azo dyes with D&C Orange 4, RCC-CCR Project 636401, Roßdorf/Germany, 7 Sep. 1999
21. H.E. Wollny; Cell mutatation assay at the thymidine kinase locus (TK +/-) in mouse lymphoma L5178Y cells with D&C Orange 4 (C.I. 15510), RCC-CCR Project 636402, Roßdorf/Germany, December 13, 1999
22. N. Honarvar; Micronucleus assay in bone marrow cells of the mouse with Orange 4 (C.I. 15510), RCC-CCR Test report, No. 741302, Roßdorf/Germany, September, 2003
23. H.E. Wollny, Skin permeability in vitro absorption through porcine ear skin with D&C Orange 4; RCC Project No. 681903; Test Report, Rossdorf, January, 24, 2003
24. M. Bracher, C. Faller, F.K. Noser, Int. J. Cosmet. Sci. 9, 223-236, 1987
25. F.K. Noser, C. Faller, M. Bracher, J. Appl. Cosmetol. 6, 111-122, 1988
26. H. Beck, M. Bracher, C. Faller, H. Hofer, Cosmetics & Toiletries, 108, 76-83, 1993
27. Cutaneous absorption of D&C Orange 4 through pig skin in vitro: the influence of carriers; Cosmital SA; year of report 2003

Opinion on Acid Orange 7

-
28. A. Tognucci; Determination of the partition coefficient (n-Octanol/water) of D&C orange 4; RCC 833905; August 30, 2002

SCCNFP references

29. Bonser GM, Bradshaw L, Clayson DB, Jull JW. A further study of the carcinogenic properties of ortho hydroxyl-amines and related compounds by bladder implantation in the mouse. Br J Cancer 10: 539-546, 1956.
30. Bonser GM, Clayson DB, Jull JW. The potency of 20-methylcholanthrene relative to other carcinogens on bladder implantation. Br J Cancer 17: 235-241, 1963

Submission II, 2005

31. Honarvar N. et al. *In vitro* dermal absorption through porcine ear skin with SC Clear + 0.5% D&C Orange 4 (Lot AJ 3559); RCC-CCR, D-Rossdorf. Study number 860802. 6 July 2005
32. Röder, A., Völkner, W. Hair. Quantification of D&C Orange 4 (Lot AJ 3559) Implementation of an Analytical Method for a Skin Dermal Absorption Assay. RCC-CCR, D-Roßdorf, Study number 860801. 12 July 2005
33. Fautz, R., Fuchs, A., Van der Walle, H., Henny, V., Smits, L.K; Contact dermatitis 2002, 46, 319-324