EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

Acid Green 25

COLIPA N° C 178

Adopted by the SCCP
during the 4th plenary meeting of 21 June 2005

TABLE OF CONTENTS

1. BACKGROUND

Acid Green 25 (COLIPA[1] n° C178) is a hair dye ingredient used in non-oxidative hair dye formulations.

Submission I for this hair dye was provided by COLIPA in September 2003.

2. TERMS OF REFERENCE

1. On the basis of currently available information, the SCCP is asked to assess the risk to consumers of Acid Green 25, when used in hair dye formulations.

2. Does the SCCP recommend any further restrictions with regard to the use of Acid Green 25 in hair dye formulations?

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Acid Green 25 (INCI name)

3.1.1.2. Chemical names

Chemical name: Disodium 2,2'-(9,10-dioxoanthracene-1,4-diyldiimino)bis(5-methylsulphonate) (IUPAC)
CAS name: Benzensulfonic acid, 2,2´-[(9,10-dihydro-9,10-dioxo-1,4-anthracenediyl) diimino]bis(5-methyl)-, disodium salt (CA Index name, 9CI)
Synonyms: 1,4-di-[(2-sulfono-4-methylphenyl)amino]-9,10-anthracenedione, disodium salt
Acid Green Anthraquinone
Alizarin Cyanine Green F
Japan Green 201
D&C Green No. 5

3.1.1.3. Trade names and abbreviations

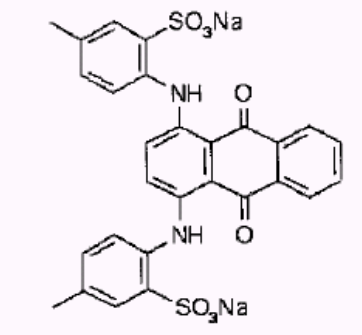
Trade name : Covacap Vert W 7103 (LCW)
Colour Index : CI 61570
COLIPA n° : C178

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

3.1.1.4.	CAS / EINECS number

CAS : 4403-90-1
EINECS : 224-546-6

3.1.1.5.	Structural formula



3.1.1.6.	Empirical formula

Formula : $C_{28}H_{22}N_2O_8S_2.2Na$

3.1.2.	Physical form

Dark green powder

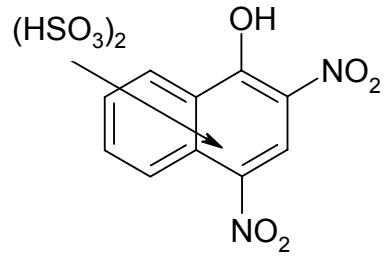
3.1.3.	Molecular weight

Molecular weight : 622.58

3.1.4.	Purity, composition and substance codes

Batch tested: Batch 33 (FDA certified Lot AJ6720)
Identification: NMR* *(spectra not yet provided)*
Content determined by NMR: 86.1%
Purity determined by HPLC (qualitative)**: >80%

Detection wavelength	% Peak area Acid Green 25	% Peak area Isomeric double sulfonated 2,4-dinitro-1-naphthol***
210 nm**	83.5	13.2
254 nm**	84.1	12.5
430 nm**	84.7	13.3



Double sulfonated 2,4-dinitro-1-naphthol

Colour content:
Citrate buffer titration: 94.8%
Spectrophotometric[a]: 94.5%
Water content: 8.2%
Volatile matter: 4.7% maximum
Sulfated ash content: 24.9%

[a] The total colour content determined by spectrophotometry may also include other organic impurities present in the Acid Green 25 - the wavelength for the colour measurement is not reported

*NMR spectrum not provided
**UV-spectrum not provided
***Method of identification of this compound is not reported

3.1.5.	Impurities / accompanying contaminants

Disulfonated 2,4-dinitro-1-naphthol: >10%
p-toluidine: <15 ppm[b]
1,4-dihydroxyanthraquinone: <0.2 %[b]
2-amino-5-methylbenzenesulfonic acid: <0.2 %[b]
1,4-diaminoanthraquinone[c]: /
Chloride content as Sodium chloride: 4.8%
Sulfate content as Sodium sulphate: 0.6%

Metal content: Pb: 0.05 ppm, As 0.1 ppm, Hg <0.1 ppm, Fe 13 ppm

[b] The analysis report is not provided. According to Annex I of Submission I and Ref. 10.
[c] Possibility of this impurity in Acid Green 25 is indicated in Ref. 10.

3.1.6.	Solubility

Water: Soluble
Acetone: 0.2% (w/v)
DMSO: 5% (w/v)

3.1.7.	Partition coefficient (Log P_{ow})

Log P_{ow} : 5.71±0.63 (acid, calculated)

3.1.8.	Additional physical and chemical specifications

Organoleptic properties	:	/
Melting point	:	235-238 °C
Boiling point	:	/
Flash point	:	/
Vapour pressure	:	/
Density	:	/
Viscosity	:	/
pKa	:	/
Refractive index	:	/

Stability and homogeneity

100 mg/ml in water containing 1% CMC: homogeneous and stable up to 7 days at 20°C
0.2% (w/v) in acetone: 8 days at room temperature
5% (w/v) in DMSO: 8 days at room temperature

General comments on analytical and physico-chemical characterisation

- A significant content (>10%) of disulfonated 2,4-dinitro-1-naphthol in Acid Green 25 is indicated by HPLC. Evidence for the characterisation of this substance is not provided.
- Quantification of potential impurities in Acid Green 25 is pending.
- According to FDA specifications Acid Green 25 may also contain mono-sulfonated D & C Green No.6 and Ext. D&C Violet No.2
- Stability of Acid Green 25 in marketed formulations is not demonstrated
- The percutaneous absorption study is performed using saline pH 3 as receptor fluid. The stability of Acid Green 25 in saline (pH 3) is not demonstrated.
- Since log P_{ow} is known to strongly depend on the pH, the reported value 5.71±0.63 is not helpful if the pH conditions used in the calculation are unknown or not related to physiological conditions and to the pH conditions of the percutaneous absorption studies.

3.2. Function and uses

Acid Green 25 is proposed for use in semi-permanent hair dye formulations as a direct dye at a maximum concentration of 0.3% in the finished cosmetic product.
Acid Green 25 is included in Annex IV of the Cosmetics Directive, and therefore, it is permitted for use in other cosmetic products

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Rat
LD_{50} >3160 mg/kg bw
LD_{50} >10000 mg/kg bw

Dog
LD_{50} >1000 mg/kg bw

Taken from reference 2 of the submission. No further details were provided.

Ref.: 2

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

No specific data from skin irritation tests in rabbits or in rodents like mice or rats are available or reported in the literature for Acid Green 25. In addition no data were noted in the literature with regard to human experience e.g. in patch tests.

An indication of the skin irritating potential of Acid Green 25 can, however, be deduced from the skin sensitisation test in guinea pigs (maximisation test, see point 3.3.3). In this test no skin irritating effects were noted when a 10 % solution of Acid Green 25 in 1 % CMC (carboxymethyl cellulose) was applied to the back skin of guinea pigs under occlusive conditions for 24 h in both the pre-test and the main study. In the pre-test a 15 % dilution produced only very mild irritation (score 1) that had completely vanished after 48 h. Furthermore, an intradermal injection of up to 5 % Acid Green 25 in CMC did not produce any irritation effects.

Although the guinea pig is not commonly used species for the determination of the skin irritation potential *in vivo* and is possibly less sensitive for skin irritation as the rabbit, the lack of any relevant skin irritation even when applied as 10 % solution and above under severe test conditions (24 to 48 h occlusive patch) is a strong indication of a low irritating effect of Acid Green 25. At the intended use concentration in hair dye formulations of 0.3 % skin irritating effects are not to be expected.

3.3.2.2. Mucous membrane irritation

Guideline	:	/
Species/strain	:	albino rabbit, strain not given
Group size	:	6 or more animals
Test substance	:	D&C Green No. 5
Batch No.	:	not given, but specifications according to FDA requirements
Dosages	:	20 mg (equal to 0.2 ml of a 10% aqueous solution)
GLP	:	not in compliance

Together with several other colorants, Acid Green 25 was investigated with regard to its eye irritation and staining properties. 0.2 ml of a 10 % aqueous solution was repeatedly applied to the conjunctival sac of one eye of each of the 6 or more animals per group for 4 weeks (twice daily on five days per week; 40 applications in total). One hour after each application, the eyes were scored for irritation according to the Draize system and for evidence of staining. In addition, scoring took also place the next day just prior to the first application of that day.

A 10 % aqueous solution of Acid Green 25 did cause a slight or spotty discoloration in the orbital tissue of some animals under the described test conditions.

One hour after the application (figures are given for day 5) a mean irritation score of 2 was determined, indicating that immediately after application mild irritant effects were present. 24 hours after the applications of the 10 % solution no indications for eye irritation were noted at any scoring throughout the entire study period.

Conclusion
Although the described study is not in line with currently recommended tests for the investigation of the eye irritating properties *in vivo* like OECD 405, the test allows an evaluation of the eye irritation potential of Acid Green 25.

As no irritation was noted under the described severe test conditions with a more than 30-times higher concentrated solution of Acid Green 25, no eye irritating effects are expected for the intended use concentrations of 0.3 % in hair dyes.

Ref.: 3

3.3.3. Skin sensitisation

Maximisation Test (Magnusson and Kligman)

Guideline	:	OECD 406 (1993)
Species/strain	:	Ibm: GO HI SPF-quality female guinea pigs (Himalayan spotted)
Group size	:	test group: 10 animals; control: 5 animals
Test substance	:	D&C Green 5 (CI 61570)
Batch No.	:	33 (lot No. AJ6720)
Concentrations	:	induction: 5 % test substance in 1 % carboxymethyl cellulose (CMC), emulsified with Freund's complete adjuvant induction: 50 % test substance in 1 % CMC, occluded challenge: 10 % test substance in 1 % CMC , occluded
GLP	:	in compliance

The dermal sensitisation potential of Acid Green 25 was evaluated by the Magnusson-Kligman Maximisation method in Ibm: GOHI SPF-quality female guinea pigs (Himalayan spotted). Based on a range-finding study, 10 animals were intradermally induced on day 1 with 0.1 ml of a 5 % dilution (w/v) of Acid Green 25 in 1 % CMC emulsified with Freund's complete adjuvant. On day 8, the animals were topically induced with a 50 % dilution of Acid Green 25 in 1 % CMC (about 0.3 g per animal). Animals were challenged on day 22 by application of about 0.2 ml of a 10 % dilution in 1 % CMC under occlusion. Approximately 24 and 48 hours after the challenge phase, the test sites were evaluated for signs of elicited sensitisation (readings after 72 hours and 7 days). The same procedures were carried out on a contemporaneous control group except that the solutions of the test article were replaced by 1 % CMC (vehicle control). No contemporaneous positive control was found; however, a positive control substance is periodically tested in the laboratory in order to document the effect of a known sensitiser in this test system.

Results

Range-finding studies

1 animal, pre-treated with 4 intradermal injections of Freund's complete adjuvant, received 0.1 ml intradermal injections of concentrations of 1, 3 and 5 % of Acid Green 25 in 1 % CMC. The injection sites were assessed 24 hours later. No reactions were noted up to a concentration of 5 %. However, a green discoloration at this dose level did not allow an evaluation for erythema. Based on these results, a 5 % dilution of Acid Green 25 in 1 % CMC was selected for intradermal induction in the main study.

A topical range-finding study with the concentrations listed in table 1 was conducted on 2 guinea pigs.

Table 1: Number of Animals Exhibiting Erythema in the Range-finding Test After Topical Application (24 **and 48 Hours After Treatment)**

Concentration	***50%***		***25%***		***15%***		***10%***	
Hours	24	48	24	48	24	48	24	48
Score	2/2	2/2	2/2	2/2	2/2	0/2	0/2	0/2

Based on the range-finding study, (50 % in 1 % CMC) was found to produce dermal irritation and was selected for the topical induction, and 10 % in 1% CMC did not produce dermal irritation and was selected as the challenge dose.

Main study

No dermal irritation was observed during the epidermal induction in the control group. As Acid Green 25 epidermally applied at 50 % in 1 % CMC causes black staining of the skin, erythema formation could not be evaluated. However, no oedema formation was noted.

Since a black discoloration was also observed after removal of the challenge patch, depilation was performed 3 hours prior to challenge reading. Skin reactions were observed neither in the control nor in the test group.

No test substance-related clinical signs of toxicity were observed.

Conclusion

Acid Green 25 had no skin sensitising effect under the conditions of the Maximisation test, in which the skin barrier is compromised. Based on these findings, Acid Green 25 is evaluated not to be a skin-sensitiser. However, the concentration of the test substance used for induction (5%) appears to be too low as no erythema was observed.

Ref.: 5

3.3.4. Dermal / percutaneous absorption

Guideline	:	OECD 428 (Draft 1996)
Tissue	:	Porcine ear skin (thickness: 300-400 µm)
Method	:	diffusion glass chambers
Test substance	:	D&C Green 5 (C.I. 61570) tested as such and as part of a commercial hair dye formulation.
Batch no	:	lot AJ6720
Concentration	:	5 mg/cm^2 (pure substance, dissolved in saline, pH 3 (adjusted), 5 mg/cm^2 (tested as part of a viscous hair dye formulation, pH 2.9-3.1)
No. of chambers	:	6 per experiment
GLP	:	in compliance

The skin absorption of Acid Green 25 was investigated on the outer skin of porcine ears (freshly obtained from the local slaughter-house, ca. 400 µm thick) with amounts corresponding to realistic use conditions. Two experiments were performed. In the first experiment 5 mg of the pure dye in 1 ml saline with an adjusted pH of 3 was applied to the skin. In the second experiment about 5 mg of the dye was applied to the skin as part of a commercial hair dye formulation (1 g hair colour gel).

The integrity of the skin was monitored throughout the study at 0, 0.5, 1, 2, 4, 6 8 and 24 h by measuring the conductivity across the skin. Intact skin usually ranges from 100-500 µS with aqueous solutions. The maximum conductivity with gross damage to the skin or without skin is 2-5 mS.

A glass diffusion chamber (donor chamber volume about 1.5 ml, skin surface 1 cm^2) was used. The receptor solution (0.9 % NaCl-solution, pH 3) was pumped through the receptor chamber by a rate of 1 -2 ml/h. Six chambers per experimental group were investigated. The donor chambers were covered by Parafilm after adding the test substance to the chamber.

Before application of the test item, receptor fluid was added to the donor chamber to check the integrity of the skin by means of conductivity measurement and to obtain the blank samples for each chamber. Only intact skin samples were used for the study. 30 min after substance application, the test item was removed by washing the skin three-times with 1 ml washing solution (10 % diluted shampoo-formulation). The washing solutions were combined and the amount of dye determined. For the remaining time of the experiment (24 h total) the donor chamber was filled with 1 ml saline solution.

Fractions of the receptor fluid were collected at 0, and for 0-0.5, 0.5-1, 1-2, 2-4, 4-6, 6-8 and 8-24 hours and stored at -20° C until analysis. The donor solution was also collected after 24 h and analysed. At termination of the experiment the skin samples (including the stratum corneum) were extracted and the dye content quantified by HPLC. Caffeine is used in the performing laboratory every 3 months as a positive control substance to demonstrate the validity of the used system.

Results
All samples/tissue extracts were analysed by HPLC, the limit of detection of the applied method was 0.15 µg/ml.

An increase of conductivity over time was observed in all chambers, but no major loss of barrier properties was noted as no abrupt change in conductivity was noted. However in the second experiment chambers 4 and 5 revealed relatively high conductivity already at begin of the study, which sharply increased and exceeded the historical control range from 1 h after application onwards. Therefore, it was concluded that the skin barrier was impaired in these two chambers after 1 h.

The mean recoveries of the test item for experiment I and II were 93.2 % ± 4.02 % and 84.6 % + 14.18 %. The higher variability of the recovery in the second experiment is most likely due to the viscous state of the formulation causing problems in application and removal of the test item. For this reason one chamber was excluded from the evaluation. The vast majority of the test item (> 99%) was determined in the combined washing solution.
No measurable permeation through the skin was noted as no Acid Green 25 was detectable in the receptor fluid for any time interval investigated. For the calculation of the penetration rate it was therefore assumed as worst case that the maximum possible concentration in the receptor fluid was 0.15 µg/ml i.e. the detection limit of the analytical method. Based on this approach, an upper limit for the penetration rate of 6.1 µg/cm^2 (0.12 % of the applied dose) and 5.8 µg/cm^2 (0.12 % of the applied dose) in experiment I and II, respectively, can be calculated, taking into account the acceptor fluid alone. Taking additionally into consideration the skin extracts (including the stratum corneum), penetration rates for Acid Green 25 of 7.9 µg/cm^2 (0.16 % of the applied dose) for the pure substance and of 17.0 µg/cm^2 (0.34 % of the applied dose) for the formulated product can be calculated from this study as worst case assumptions.

Conclusion
Under the described test conditions, a low skin penetration rate for Acid Green 25 was obtained as no detectable amounts were noted in the receptor fluid. In a worst case scenario, the penetration rate was therefore calculated based on the determined LOD of 0.15 µg/ml for the applied HPLC method. A penetration rate of 7.9 µg/cm^2 (about 0.16 % of the applied amount) is obtained if the test item is applied as pure substance at pH 3 and the extract of the skin (including the stratum corneum) is considered to be absorbed. A slightly higher penetration rate of 17.0 µg/cm^2 (0.34 % of the applied dose) is obtained if the dye is applied as part of a commercial hair colour gel.

Ref.: 6

Comments
The content of the dye in the receptor fluid and skin extracts was performed by HPLC employing 450 nm as the detection wavelength. From the spectrum of Acid Green 25 reported in the literature, the absorbance of the dye at this wavelength is minimal. For this reason the dye might not have been detected in the receptor fluid. Furthermore, the use of wrong wavelength for the detection of the dye may also be responsible for not finding any difference between the amounts of dye penetrated through the intact and impaired membrane. A dermal absorption study using a receptor fluid at pH 3, is not giving physiological data and is therefore not considered to be relevant here.

In conclusion, the study is inadequate.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) oral / dermal / inhalation toxicity

No data submitted

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Guideline	:	OECD 408
Species/strain	:	Wistar rats, HanIbm: WIST(SPF)
Group size	:	10 animals per sex and dose, no recovery group
Test Substance	:	Acid Green 25 in bi-distilled water containing 1 % carboxymethyl cellulose
Batch No	:	33 (lot No. AJ 6720)
Dose level	:	100, 300, 1000 mg/kg bw/day
Route	:	Oral, gavage
Exposure period	:	13 weeks
GLP	:	in compliance

In a 14-day dose range finding study, no treatment-related effects were noted up to the limit dose of 1000 mg/kg bw/day, except discolouring of faeces, limbs, skin and testes (high dose group). Based on the results of this study, 100, 300 and 1000 mg/kg bw were chosen for the main study.

In the main study, Acid Green 25 dissolved in bi-distilled water containing 1% CMC was administered daily to groups of 10 males and 10 female Wistar HanIbm rats by gavage in a total volume of 10 ml/kg bw over a period of 13 consecutive weeks. In addition, an equally sized control group received the same dose volume of the vehicle. Homogeneity and the stability of the test solutions were evaluated.

Mortality was checked twice daily, clinical signs were recorded once daily. Detailed clinical observations, individual body weights and food consumption were recorded weekly. An ophthalmological examination was performed in all animals before treatment and at week 13 for the control and high dose group only.

During week 13 relevant parameters of a functional observational battery (modified Irwin screen test) were evaluated as well as grip strength and locomotor activity. Clinical laboratory investigations (haematology, blood/clinical biochemistry and urinalysis) were performed at the end of the treatment period. All animals were subjected to a detailed necropsy and a number of organs (adrenals, brain, heart, kidneys, liver, ovaries, testes, spleen, thyroid and thymus) were weighed and several tissues and organs were fixed and stored for further examinations if required.

Results
No substance-related mortalities and significant clinical signs were noted in the treated animals. Discoloration of faces and gastrointestinal tract was noted in all treated groups.
No test item related findings were noted in the weekly performed clinical observations. The functional observational battery as well as grip strength and locomotor activity measurements in week 13 did not reveal test item related effects.

No effects were noted with regard to body weight, body weight development and food consumption during the study period. At necropsy, effects on organ weights were noted for kidney only. The absolute and/or the relative kidney weight increased for both sexes at 300 and 1000 mg/kg bw/day, being more pronounced at 300 mg/kg bw/day. The kidney/brain ratio also revealed statistically significant increases at both doses or at 300 mg/kg bw/day only. A detailed analysis of the individual data revealed no clear dose response and that effects were seen for one parameter only (absolute weight increased, relative not, etc.) Furthermore, the brain weights (absolute and relative) in females were slightly decreased compared to control controls for all treated groups, without revealing a dose-response relationship.

Some changes in haematology parameters were found at the highest dose level. Urinalysis revealed a significant increase in urine volume in males of the high dose group. No abnormalities were noted in the histological investigation or with regard to markers of kidney physiology.

Comment
Due to the effects on absolute and relative kidney weight at 300 mg/kg bw/day and above, a no observed effect level (NOEL) of 100 mg/kg bw/day was deduced.

Ref.: 4

3.3.5.3.	Chronic (> 12 months) toxicity

See 3.3.7

3.3.6.	Mutagenicity / Genotoxicity

3.3.6.1.	Mutagenicity / Genotoxicity *in vitro*

Bacterial gene mutation assay

Guideline	:	OECD 472
Species/strain	:	*S. typhimurium*, TA98, TA100, TA1535, TA1537; *E. coli*, WP2uvrA
Replicates	:	Two independent tests with and without metabolic activation
Test substance	:	Acid Green 25 (C 178); D&C Green 5 (C.I. 61570) in deionised water
Batch No.	:	33 (purity: 95%)
Lot No.	:	AJ 6720
Concentrations	:	33 - 5000 µg/plate without and with metabolic activation
GLP	:	in compliance

Acid Green 25 (C 178) has been investigated for the induction of gene mutation in *Salmonella typhimurium* and *E. coli*. Liver S9 fraction from rats induced with Phenobarbital and ß-Naphthoflavone was used as the exogenous metabolic activation system. Negative and positive controls were in accordance with the OECD guideline.

Results
In one experiment, moderate toxic effects (reduced number of revertants) were seen in strain TA 1535 at 5000 µg/plate without S9 mix and from 1000 up to 5000 µg/plate with S9mix and in

strain TA 1537 at 2500 and 5000 µg/plate without S9 mix. No induction of mutations was measured in any of the experiments. Negative and positive controls gave the expected results.
Acid Green 25 (C 178) is not mutagenic in the bacterial gene mutation assay.

Ref.: 7

***In vitro* mammalian cell gene mutation test**

Guideline	:	OECD 476
Cells	:	L5178Y mouse lymphoma cells (TK+/-)
Replicates	:	2 independent tests Experiment I: 4 h treatment with and without S9 mix Experiment II: 24 h treatment without S9 mix
Test substance	:	Acid Green 25 (C 178); D&C Green 5 (C.I. 61570) in deionised water
Batch No.	:	33 (purity: 95%)
Lot No.	:	AJ 6720
Concentr. Tested	:	78.1 - 1250 µg/ml without metabolic activation (expt. I) 312.5 – 2500 µg/ml with metabolic activation (expt. I) 312.5 - 2500 µg/ml without metabolic activation (expt. II)
GLP	:	in compliance

Acid Green 25 (C 178) has been investigated for induction of gene mutations at the TK-locus in L5178Y mouse lymphoma cells after exposure for 4 hours without and with metabolic activation and for 24 hours without metabolic activation. Liver S9 fraction from Phenobarbital and ß-Naphthoflavone-induced rats was used as the exogenous metabolic activation system. Test concentrations were based on results from a pre-experiment on toxicity. The level of toxicity was between 70 and 10% relative total growth for the highest concentration in the main experiments. Negative and positive controls were in accordance with the OECD guideline.

Results
In the second experiment without S9 mix and treatment for 24 hours, a single isolated increase in the number of mutant colonies was measured at 1250 µg/ml in one out of two parallel cultures. No increased mutant frequencies were observed at a higher concentration (2500 µg/ml) in the same test. No mutagenic effect was observed in the first experiment. Therefore, the isolated positive effect does not have biological significance. Under the experimental conditions used, Acid Green 25 (C 178) has to be considered non-mutagenic in mammalian cells (L5178Y mouse lymphoma cells) *in vitro*.

Ref.: 8

3.3.7. Carcinogenicity

Mouse
Oral treatment (presumably in the diet) at 0.05, 0.5 and 2.0 % did not cause relevant substance-related toxic and carcinogenic effects.

Rat
Oral treatment (presumably in the diet) at 0.1, 0.25 and 1.0 % did not cause substance-related effects.

Rat
Oral treatment (presumably in the diet) at 0 and 1.0 % for 2 years induced discoloration of eyes, serum, intestine and body fat as well reduction of liver weight.

Dog
Oral treatment (presumably in the diet) at 0 and 1.0 % for 2 years induced no substance-related effects with the exception of discoloration of fat and gall bladder content.

Taken from reference 2 of the submission. No further details were provided.

Ref.: 2

3.3.8. Reproductive toxicity

3.3.8.1. Two generation reproduction toxicity

No data submitted.

3.3.8.2. Teratogenicity

No data submitted. The applicant announced a teratogenicity study.

3.3.9. Toxicokinetics

No data submitted

3.3.10. Photo-induced toxicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

No data submitted

3.3.13. Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Not applicable

3.3.14. Discussion

The submitted data on physico-chemical properties of Acid Green 25 is incomplete. No data is provided for the chemical identification of an impurity that is present in >10% in Acid Green 25.

Acid Green 25 is evaluated not to be an irritant to the skin and eyes. It did not induce sensitisation in one Guinea pig experiment.

The submitted *in vitro* dermal absorption study of Acid Green 25 is inadequate.

The 13 week oral toxicity study in rats revealed a NOEL of 100 mg/kg bw/day. No data on reproduction toxicity were submitted.

Acid Green 25 (C178) has been tested for mutagenicity in two appropriately performed *in vitro* tests. It did neither induce gene mutations in bacteria nor in cultured mammalian cells. Since the mouse lymphoma assay (MLA) also detects clastogenic activity of a test compound, the negative result suggests that Acid Green 25 does not induce structural chromosome aberrations. However, an additional *in vitro* test specifically detecting chromosomal aberrations (preferable an *in vitro* micronucleus test which detects clastogenic and aneugenic effects) should be performed to exclude a mutagenic potential of Acid Green 25.

4. CONCLUSION

The SCCP is of the opinion that the information submitted is insufficient to assess the safe use of the substance. Before any further consideration, the following information is required:

- complete information on the >10% impurity;
- Data on exposure from other cosmetic products;
- a percutaneous absorption study according to the Notes of Guidance;
- Data on teratogenicity;
- An additional *in vitro* test specifically detecting chromosomal aberrations (preferably an *in vitro* micronucleus test which detects clastogenic and aneugenic effects) should be performed.

5. MINORITY OPINION

Not applicable

6. REFERENCES

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