Scientific Committee on Consumer Safety

SCCS

OPINION ON

HC Yellow No. 17
(B121)

The SCCS adopted this Opinion at its 10th plenary meeting

on 25 June 2015

Revision of 15 December 2015

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.).

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This opinion has been subject to a commenting period of eight 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.

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1. BACKGROUND

Submission I on the hair dye HC Yellow No. 17 (INCI) (No B121) CAS No 1450801-55-4 with the chemical name Di-[2-[4-[(E) -2-[2,4,5-trimethoxyphenyl] vinyl]pyridinin-1-ium] butanoyl] aminoethyl]disulfanyl dichloride was transmitted by Cosmetics Europe in April 2014.

The new ingredient HC Yellow No. 17 (B121) is planned to be used in non-oxidative hair colouring products at concentrations of up to 0.5%.

2. TERMS OF REFERENCE

(1) In light of the data provided, does the SCCS consider HC Yellow No. 17 (B121) safe when used in non-oxidative hair colouring products at concentrations of up to 0.5%?

(2) Does the SCCS have any further scientific concerns with regard to the use of HC Yellow No. 17 (B121) in cosmetic products?

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

HC Yellow No. 17 (INCI)

3.1.1.2 Chemical names

Di-[2-[4-[(E)-2-[2,4,5-trimethoxyphenyl]vinyl]pyridinin-1-ium]butanoyl]aminoethyl]disulfanyl dichloride
Pyridinium, 1,1'-[dithiobis[2,1-ethanediylimino (4-oxo-4,1-butanediyl)]] bis [4-[(1E)-2-(2,4,5-trimethoxyphenyl)ethenyl]-, chloride (1:2) (CAS Name)

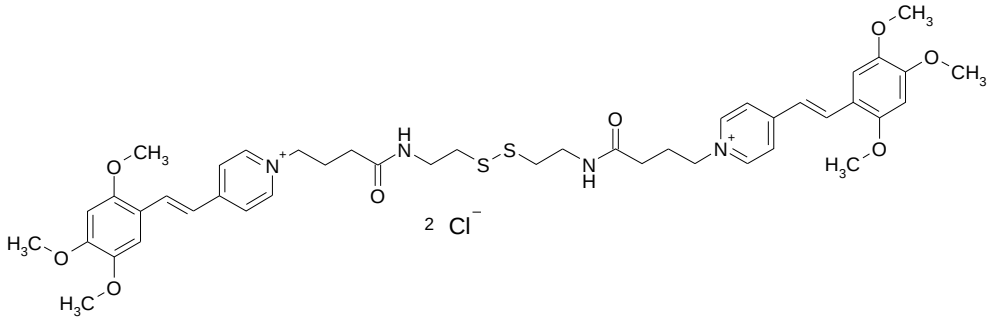
3.1.1.3 Trade names and abbreviations

Trade names: Vibracolor Golden Yellow
Other codes: E212289 / BCF-52281

3.1.1.4 CAS / EC number

CAS: 1450801-55-4
EC: /

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

$C_{44}H_{56}N_4O_8S_2$, 2Cl

3.1.2 Physical form

Dark red powder

3.1.3 Molecular weight

Molecular weight: 903 g/mol

3.1.4 Purity, composition and substance codes

All batches were characterised using conventional analytical techniques: Infra-red and NMR spectrometry, Mass Spectrometry, UV_Vis spectrometry, HPLC and GC elemental analysis. The results of the analytical study of Vibracolor Golden Yellow Batches is listed in the table below.

Analytical test	**Batch 5**	**Batch LR1**	**Batch Op. 2/13 Crude UK***
Aspect	Dark red powder		Red powder
HPLC Titre determined against R0074777A002L001 considered as 85.7 % pure	75.4 % w/w	83.5 % w/w	85.4 % w/w
Impurities (> 0.1%) content determined against: R0074777A002L001 considered as 100 % pure	For *these two batches impurities were identified by a number (Imp. 1,2,3...- See left column)*		*For this batch impurities were identified by their retention times (See below)*
Imp. 1	*0.27 % w/w*		
Imp. 2	0.15 % Area %		0.4 % Area % (11.9 min)
Imp. 3+4	0.49 % w/w	0.32 % w/w	0.1 % Area % (14.5 Min)
Imp. 5	0.19 % w/w		0.1 % Area % (17.6 min)
Imp. 6	0.53 % w/w		1.3 % Area % (18.9 min)
Imp. 7	0.33 % w/w	0.16 % w/w	0.7 % Area % (19.2 Min)
Imp. 8	0.15 % w/w		0.2 % Area % (19.5 Min)
Imp. 9	0.45 % w/w		0.3 % Area % (22.4 Min)
2, 4, 5-trimethoxybenzaldehyde	0.12 % w/w	0.12 % w/w	0.29 % w/w
Imp. 10	0.18 % w/w		0.3 % Area % (24.1 min)
Imp. 11+12	0.16 % w/w		
Imp.13+14+15+16	0.53 % w/w	2.39 % w/w	
Imp. 17	0.19 % w/w	0.52 % w/w	
Imp. 18	0.12 % w/w		
Imp. 21	0.51 % w/w	0.16 % w/w	
Imp. 22	0.37 % w/w		
Imp. 23	0.31 % w/w	0.33 % w/w	
Sum of impurities > 0.1%	5.05 % w/w	4.0 % w/w	3.69 % (3.4 % Area % - *0.29 %*
Sum of Impurities < 0.1%	0.5 % Area %	0.13 % Area %	0.4 % Area %

Analytical test	**Batch 5**	**Batch LR1**	**Batch Op. 2/13 Crude UK**
Water content (%w/w)	11.8	9.7	4.4
Residual solvents and reagents content µg/g)			
Ethanol	<1000 ND	<1000 ND	<500
Toluene	<500 ND	<500 ND	<100
2-Propanol	<1000 ND	<1000 ND	50 000 (NMR)
Ethylacetate	<1000 ND	<1000 ND	1300
4-Picoline	<100 ND	<100 ND	96
Pyrrolidine	27000 (2.7 % w/w)	300	1900
HCl		3000 (estimated - based on elemental analysis)	
Acetic acid	39000 (3.9 % w/w) (Based on elemental analysis)	3200 (0.32 % w/w) (Based on elemental analysis)	7500 (NMR) (0.75 % w/w)
Counter ion (%w/w)			
Acetate	0.6 (0.1mol/mol) as counter ion	No acetate as counter ion	0,6
Chloride (Theoretical: 7.8)	6.1 (1.9mol/mol)	7.3 (2mol/mol)	6,9 (2mol/mol)
Nitrosamines µg/kg)			
Total N-Nitroso compounds (expressed as NO)	2980**	<50	<50
Heavy metals and ashes			
Heavy metals	See 3.1.5		
Ashes (% w/w)	<0.1%	0,9	--
Total %w/w	99.35	98.9	99.97

* Certificate of analysis was provided for this batch

HPLC profile of batches 5 and LR1 were not comparable with respect to impurities. More impurities were detected in batch 5. On the basis of MS/MS, most of the 1-23 impurities were proposed to be related to the active ingredient and/or fragments of these.

** The applicant declared that this high value is due to the re-crystallization process on a lab batch. This process will not occur on the industrialised batches. Total N-Nitroso compounds content is planned to be < 50 µg/kg.

SCCS comment

No information about the reference standard R0074777A 002L001 used for the determination of purity and impurities was provided, except that for the purity determination the applicant considered it as 85.7% pure and for the impurity determination it was considered 100% pure. No documentation was provided.

3.1.5 Impurities / accompanying contaminants

See 3.1.4
Heavy metal content:

Element	Vibracolor Golden Yellow Experimental values (mg/kg)	
	Batch 5	Batch LR1
Aluminium	<5	<26
Antimony	<1	<1
Arsenic	<1	<1
Barium	<5	<5
Calcium	<50	132
Cadmium	<1	<1
Cobalt	<5	<5
Chromium	<5	<5
Copper	<5	<5
Iron	10	18
Lead	<1	<1
Mercury	<1	<1
Manganese	<5	<5
Molybdenum	<5	<5
Nickel	<5	<5
Potassium	<50	<50
Palladium	<1	<1
Phosphorus	<50	<50
Selenium	<5	<5
Sodium	67	3332
Titanium	<5	<5
Tin oxide	<5	<5
Vanadium	<5	<5
Zinc	10	6

3.1.6 Solubility

Solubility of Vibracolor Golden Yellow Batch LR1at (23±2 ^{0}C) evaluated according to the European Pharmacopoeia protocol, 5.11.

Ultra pure water: 0.1-1 g/L (pH of the solubilised fraction of 1% w/w suspension = 7.7)

Absolute ethanol: 1-10 g/L

DMSO 50-100 g/L, Corn Oil < 0.1 g/L

SCCS comment
Water solubility has not been determined according to the EC Method A.6.

3.1.7 Partition coefficient (Log P_{ow})

Log P_{ow}: /

The applicant declared that "*Log $P_{o/w}$ could not be measured in our conditions (potentiometry) due to the too high hydrophilicity of the molecule. It is evaluated inferior to -2, which is in agreement with ClogP prediction (-2.7)*".

SCCS comment
EC Method A.8 was not used for the determination of Log P_{ow}.

3.1.8 Additional physical and chemical specifications

Melting point:	73-80°C
Boiling point:	
Flash point:	
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	/
Refractive index:	/
pH:	/
UV_Vis spectrum (200-800 nm):	Four maxima were exhibited at 428nm, 334nm, 285nm and 265nm. Fluorescence spectra of Vibracolor Golden Yellow Batches 5 and LR1 were comparable

3.1.9 Homogeneity and Stability

10 mg/mL and 200 mg/mL dose formulations of HC Yellow No. 17 in corn oil were shown to be homogeneous and stable (variation < 10%) up to 8 days at room temperature.

Ref. 1

Stability of HC Yellow No. 17 in typical hair dye formulations containing 0.5% HC Yellow No. 17 was performed during the skin penetration study. Twenty-four (24) hours after application, the penetration, mass balance and distribution of HC Yellow No. 17 were determined by measuring its concentration in various skin compartments. Mean recovery of the applied test material (mass balance) was at 96.4%.

Ref. 5

General Comments to physico-chemical characterisation

No information about the reference standard used for the determination of HC Yellow No. 17 was provided except that it was considered to be 85.7% pure. EC Methods were not used for the determination of water solubility and Log P_{ow}. EC number of HC Yellow No. 17 has not been provided.

3.2 Function and uses

The ingredient HC Yellow No. 17 is intended to be used in non-oxidative hair colouring products at a maximum concentration of 0.5%.

3.3 Toxicological Evaluation

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

No acute oral toxicity studies were submitted with HC Yellow No. 17. However, no deaths were observed in the subchronic (13-week) oral toxicity study performed in rats as well as in the developmental toxicity study in rats at dose levels of 100, 300 and 1000 mg/kg bw/d (Ref. 6 and Ref. 10).

3.3.1.2 Acute dermal toxicity

/

3.3.1.3 Acute inhalation toxicity

/

3.3.1.4 Acute intraperitoneal toxicity

/

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Guideline:	OECD 431 ECVAM validated protocol (ESAC statement 2007 on the validity of *in vitro* test for skin irritation)
Test system:	Human Episkin small model (0,38cm²)
Replicates:	3 different tissue batches
Test substance:	HC Yellow N°17
Test batch:	BCF52281-LR1
Purity:	83.50%
Test item:	Undiluted
Vehicle:	None
Dose level:	10 mg
Treatment period:	15 minutes
Post-treatment incubation time:	42 hours
Positive control:	10µl of 50mg/ml aqueous solution of Sodium Dodecyl Sulfate
Negative control:	10µl of PBS+
Direct interaction with MTT:	Negative
Colouring of epidermis:	Positive
GLP:	In compliance

Study period: 15 October 2013 - 14 November 2013

The test item, positive and negative controls were tested in triplicate. One additional negative control which followed the same treatment as the negative control except the MTT incubation period was added. Three additional tissues were used and followed the same treatment with the test item as the other tissues, except for the MTT incubation period. These tissues as well as the additional negative control were used as additional specific controls in order to quantify the non-specific colour due to the colouring of chemical interactions with the tissue. 10 mg of the test substance was applied onto the epidermis. After a 15 minutes treatment period at room temperature, tissues were rinsed with 25 ml PBS+. The epidermis were then transferred on 2 ml/well of fresh maintenance medium for 42 ± 1 hours at 37°C, 5% CO_2 and 95% humidity. Maintenance culture media were kept frozen at -20°C for further IL-1α measurements. After the 42 hour incubation period, plates containing the treated epidermis were shaken and each epidermis unit was transferred to another 12-well plate containing 2 ml/well of dye solution (0.30 mg/ml MTT in assay medium), except for the negative control and the test item-treated epidermis without MTT which were transferred in another 12-well plate containing 2 ml/well fresh assay medium. After a 3 hour ± 15 minutes incubation period at 37.0 ± 1.5 °C, 5.0 ± 0.5% CO_2 and 95% humidity, a biopsy of the entire epidermis was taken. For all tissues with the test item, the superficial epidermis layer (containing most of the remaining colour) was removed and discarded. The epidermis was separated from the collagen matrix and both were transferred into a tube containing 500 µl acidified isopropanol. Formazan crystals were extracted during the weekend at 5°C protected from light. After homogenisation by using a vortex, the optical density was measured at 570 nm versus acidified isopropanol as blank and the % cell viability was calculated.
IL-1α released in the culture medium was determined by a classic quantitative sandwich immunoassay technique. Monoclonal specific IL-1α antibodies were pre-coated onto micro-plates. 200 µl of standards or samples were added in the wells enabling IL-1α to bind to immobilised antibody. After washing, an enzyme-linked polyclonal antibody specific to Il-1α was added to the wells. A substrate solution was added and the intensity of the colour developed was measured at 450 nm.

Results
The mean viability for undiluted HC Yellow 17 was 85.1 ± 10.3 % and the mean IL-1α release was 18.1 ± 7.2 pg/ml.

Conclusion
Under the conditions of this study, it can be concluded that undiluted HC Yellow 17 is non-irritant after dermal application.

Ref. 2

3.3.2.2 Mucous membrane irritation / Eye irritation

Guideline:	OECD 437 (September 2009)
Test material:	Bovine cornea
Replicates:	3 corneas per condition
Test item:	E212289
Test batch:	BCF52281-LR1
Purity:	97.4% at 428 nm, 96.7% pure at 265 nm
Test item:	20% (w/w) in 0.9% (w/v) NaCl
Treatment period:	4 hours
Post-treatment incubation time:	None
Positive control:	20% (w/v) Imidazole in 0.9% (w/v) NaCl
Negative control:	0.9% (w/v) NaCl
GLP:	In compliance
Study period:	22 May 2013 - 29 November 2013

Bovine eyes (from cattle aged less than 12 months) were collected at slaughterhouses and prepared within 4 hours of collection. 750 ± 8 µl of the test item E212289 diluted at 20% (w/w) in 0.9% (w/v) NaCl was applied onto the cornea (category: solid non surfactant). The test item remained in contact with the isolated cornea for 4 hours ± 10 minutes. Six corneas per condition were used. At the end of the contact period, the corneas were rinsed and prepared for measurement of opacity (changes in light transmission). Three of these corneas were further used to measure the permeability (evaluation of transfer of 5 mg/ml fluorescein through the cornea by measuring the optical density at 490 nm of the media in the ocular posterior compartment). The remaining three corneas were kept for histological analysis. The corneal score, which is the combination of opacity and permeability, was then calculated. Negative and positive control substances were tested according to the same experimental conditions.

Results
The score obtained for E212289 diluted at 20% (w/w) in NaCl 0.9% after 4-hour contact was 0. The histological analysis showed no evidence of topical irritation.

Conclusion
Under the experimental conditions of this study, the test item designated as E212289 applied diluted to 20% (w/w) in 0.9% (w/v) NaCl, is not classified corrosive or severe irritant for the isolated bovine cornea, after 4 hours of contact.

Ref. 3

SCCS comment
On the basis of the results obtained in the BCOP study, it can be concluded that HC Yellow No. 17 at 20% (w/w) in physiological saline is not a strong eye irritant. This does not, however, exclude a mild or moderate eye irritancy potential. Under the conditions of this study, an eye irritation potential of HC Yellow No. 17 at 20% (w/w) in physiological saline cannot be excluded. Considering that the maximum intended concentration of HC Yellow No. 17 in a hair dye product is 0.5% (w/w) and no severe eye irritation potential is observed in the BCOP test for 20% (w/w) HC Yellow No. 17, it can be assumed that eye irritation will be of limited concern.

3.3.3 Skin sensitisation

Local Lymph Node Assay (LLNA)

Guideline:	OECD 429
Species/strain:	female CBA/JRj mice
Group size:	Sixty-four (64) mice
Test substance:	HC Yellow No. 17.
Batch:	E212289 BCF-52281/5
Purity:	(relative purity 93.7% at 265 nm, 95.5% at 428 nm)
Vehicle:	the vehicle (DMF) alone and the relevant vehicle for HCA (AOO)
Concentration:	1, 5, 10, 25 and 50% (w/v) in dimethylformamide (DMF)
Positive control:	alpha-hexylcinnamaldehyde (HCA) at 25% (v/v) in a mixture acetone/olive oil (4/1, v/v), thereafter designated as AOO
GLP:	yes
Study period:	Mar 2012-Nov 2012

In order to evaluate the skin sensitisation potential of the item E212289, two consecutive studies based on the Local Lymph Node Assay were conducted. The difference between the studies was the concentrations of the item solutions that were assessed. Two studies were necessary to get conclusive results of the sensitisation potential of HC Yellow No. 17.

In both studies CBA/ female mice were used. Both ears were treated with HC Yellow No.17 at different concentrations diluted in N,N-Dimethylformamide (DMF). In the first experiment, the item solution was tested at concentrations of 50%, 25%, 10%, 5% and 1% w/v. These concentrations were selected according to a previous study of solubility showing 50% as the maximum concentration of HC Yellow No.17 possible when using DMF as vehicle.
The concentrations of the solutions tested in the second experiment were 2,5%, 1%, 0,5%, 0,25% and 0,1% w/v. The solutions were applied for three consecutive days.
Alpha-hexylcinnamaldehyde was used as positive control.

Results
Mild local irritation was observed using the highest concentrations of HC Yellow No.17. SI's higher than 3 were observed for the test concentrations at and above 1 (4.3 for 1% to 19.7 for 50%) suggesting a high sensitising potency. Because an EC3 could not be calculated, the second experiment with lower concentrations of HC Yellow No.17 was conducted.
Based on the calculated EC value of 0.7% once solutions of 2,5%, 1%, 0,5%, 0,25% and 0,1% w/v were used, it was concluded that HC Yellow No.17 has sensitising potential.
The validity criteria were fulfilled in both. SI values of 9.1 and 6.1 were obtained with the positive control HCA in the first and second experiments, respectively.
The lymphoproliferative responses were observed at all concentrations tested in the first experiment. It was attributed to delayed contact hypersensitivity in the absence of excessive local irritation.

Conclusion
Under the conditions of this study, HC Yellow No. 17 induced delayed contact hypersensitivity. According to the calculated EC_3 value (0.7%), HC Yellow No. 17 was considered to have a sensitising potential.

Ref. 4

SCCS comment
Under the conditions used in this study, HC Yellow No. 17 is considered as a strong sensitiser capable of inducing delayed contact hypersensitivity.

3.3.4 Dermal / percutaneous absorption

Guideline:	OECD 428
Species/strain:	Human dermatomed abdominal skin
Membrane integrity:	Checked by electrical resistance, at least 10 kOhm
Group size:	12 skin samples from 4 human donors
Method:	Dermatomed thawed skin mounted on diffusion cells, and exposed to radiolabelled dye for 20 minutes.
Test substance:	[methoxymethyl-^{14}C]-Dye C
Batch:	[14C]-E212289 and CFQ41700
Purity:	relative purity 97.4% at 428 nm and 99.5% radiochemical pure
Test item:	hair dye formulation containing 0.5% HC Yellow 17
Dose volume:	20 mg/cm2 formulation, corresponding to 100 µg/cm^2 dye
Receptor fluid:	PBS
Method of Analysis:	Liquid scintillation counting
GLP:	yes
Study period:	March 2013 to July 2013

Human abdominal skin samples were obtained from four different female donors from a tissue bank. The membranes were stored frozen, at approximately -20° C, on aluminum foil until use.
Skin samples were dermatomed (400 µm in thickness) and mounted onto diffusion cells, using degassed phosphate buffered saline (PBS) as the receptor fluid. Membrane integrity was determined by measurement of the electrical resistance across the skin membrane. Membranes with a measured resistance of <10 kΩ were regarded as having a lower integrity than normal and not used for exposure to the test materials. Twelve intact skin membranes (from four human donors) were used and skin was maintained at approximately 32°C. Preliminary investigations showed that HC Yellow No. 17 bound to the glassware, which was therefore treated with 5% dimethyldichlorosilane in toluene. This treatment yielded a good recovery rate.
A typical hair dye formulation containing 0.5% HC Yellow No. 17 was tested. About twenty (20) mg/cm² of this formulation (corresponding to a nominal dose rate of 100 µg/cm² of HC Yellow No. 17) was applied to the skin surface and left for 20 minutes. After this time period, the remaining formulation on the skin surface was removed using a standardised washing procedure, simulating use conditions. Twenty-four (24) hours after application, the percutaneous absorption of HC Yellow No. 17 was estimated by measuring its concentration by liquid scintillation counting in the following compartments: skin washes, *stratum corneum* (isolated by tape strippings), living epidermis/dermis, unexposed skin and receptor fluid.

Results
All diffusion cells yielded data that could be analysed and the mean recovery rate was good at 96.4%. The HPLC analysis of the hair dye formulation performed following the dosing procedure and 24 hours post application was 99.2 and 99%, respectively, confirming that the formulation was stable for a 24-hour period.
Most of the HC Yellow No. 17 applied on the skin surface was removed with the skin wash at 20 minutes (96.3%).
The mean amount of HC Yellow No. 17 considered as systemically available was estimated as follows (sum of amounts measured in living epidermis/dermis and receptor fluid): 0.021 ± 0.013 µg.eq/cm^2 (0.019 ± 0.012% of the applied dose).

Conclusion
The results obtained in this study indicate that HC Yellow No. 17 present at 0.5% in a typical hair dye formulation penetrated through human dermatomed skin at an extremely slow rate. The amount of HC Yellow No. 17 considered as absorbed was estimated to be at most 0.021 ± 0.013 µg.eq/cm^2 corresponding to 0.019 ± 0.012% of the applied dose.

Ref. 5

SCCS comment
SCCS considers that the mean plus 2 standard deviations (0.047 µg/cm^2) should be used for MOS calculation because of the short exposure time.

3.3.5 Repeated dose toxicity

3.3.5.1 Repeated Dose (14 days) oral toxicity

3.3.5.2 Sub-chronic (90 days) toxicity (oral)

Guideline: OECD 408
Species/strain: Wistar rats – HsdCpb: WU rats

Group size:	10 males and 10 females per dose group
Test substance:	E212289
Batch:	BCF52281-LR1
Purity:	83.5 % w/w, 97.4 Area % (at 428 nm), 96.7 Area % (at 265 nm)
Vehicle:	Corn oil (suspension)
Dose levels:	0, 100, 300 or 1000 mg/kg bw/d
Dose volume:	5 mL/kg bw
Route:	oral
Administration:	gavage
GLP:	in compliance
Study period:	07 December 2012 - 07 March 2013

The subchronic toxicity of HC Yellow No. 17 was investigated in a 13-week oral (gavage) toxicity study in rats. A total of 40 males and 40 female rats were randomly allocated to four groups and each group comprised of 10 rats/sex. HC Yellow No. 17 was administered at 0, 100, 300 or 1000 mg/kg bw/d. These dose levels were selected on the basis of the results obtained in a preliminary 14-day study where no adverse effects were observed up to 1000 mg/kg bw/d (Ref. 11).
All rats were observed for clinical signs and mortality daily. Bodyweights and food consumption were measured during the course of in-life phase of the experiment. Functional observation tests were performed during week 13. Blood samples were collected for clinical chemistry and haematology at the end of the study. All rats were sacrificed and subjected to gross examination. Forty four organs as specified in the study plan were collected, weighed and preserved. Histopathological examination was carried out on all preserved tissues from all rats of the vehicle control and the high dose group. In addition, all gross lesions from all rats were examined microscopically.

Results
No mortality was observed neither in the test item treated groups nor in the vehicle control group. Slight salivation was reported in all test item treated groups to a dose-dependent degree, starting 5-10 minutes post-dosing and persisting up to 20 to 45 minutes post-dosing. The salivation was considered non-adverse but due to the oral administration via gavage. At 300 and 1000 mg/kg bw/d, reddish brown coloured faeces (colour matching with test item) were observed during the treatment period which was considered to be due to the colour of the test item. Bodyweight, net bodyweight gain and food consumption of rats were unaffected by the treatment at all dose levels and were similar to that of the vehicle control group. Ophthalmologic examination did not reveal any ocular abnormalities in any dose groups compared to the vehicle control group. Neurological examination did not reveal any treatment-related abnormalities in the treated animals and were similar to the vehicle control group. No relevant treatment-related changes were detected with respect to haematology, coagulation, clinical chemistry and urinalysis parameters of the animals treated with the test item compared to the vehicle control animals. No treatment-related changes were detected regarding terminal fasting body and organ weights, gross and microscopic examination compared to the vehicle control group. Gross pathological examination showed reddish brown contents in the caecum of all rats at 1000 mg/kg bw/d, which was considered to be due to the colour of the test item present in the caecum. Histopathological examination showed no relevant treatment-related lesions in males and females of the 1000 mg/kg bw/d group compared to the vehicle control group.

Conclusion
Under the conditions of this study, the "No Observed Adverse Effect Level (NOAEL)" of E212289 in Wistar rats is defined at 1000 mg/kg bw/d.

Ref. 6

SCCS comment
It cannot be ruled out that the statistically significant decrease in the number of reticulocytes (17% at 1000 mg/kg bw/d in males; 25 and 26% at 100 and 300 mg/kg bw/d in females) is substance related. However, no clear dose-response relationship was observed and no further changes indicating haematotoxicity were recorded. The increase of alanine aminotransferase (38%) and the decrease of inorganic phosphorous (20%) at 1000 mg/kg bw/d were also isolated findings of unclear toxicological significance.

3.3.5.3 Chronic (> 12 months) toxicity

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Bacterial reverse mutation test (Ames)

Guideline:	OECD 471
Test system:	*Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537
Replicates:	2 experiments
Test substance:	HC Yellow No. 17
Batch:	E212289 BCF-52281/5
Purity:	(relative purity 95.5 % pure at 428 nm)
Concentrations:	Experiment I: ±S9-mix: 5, 15.81, 50, 158.1, 500, 1581 and 5000 µg/plate Experiment II: 51.2, 128, 320, 800, 2000 and 5000 µg/plate for strains TA98 and TA100 both in the presence and absence of S9 mix, and for strain TA1535 in the absence of S9 mix; 8.192, 20.48, 51.2, 128, 320, 800 and 2000 µg/plate for strain TA102 in the absence of S9 mix, and for strains TA102, TA1535 and TA1537 in the presence of S9 mix 20.48, 51.2, 128, 320, 800, 2000 and 5000 µg/plate for strain TA1537 in the absence of S9 mix
Treatment:	Experiment I Direct plate incorporation test (SPT), ±S9-mix. Experiment II Direct plate incorporation test without S9-mix and Pre-incubation test + S9-mix
Vehicle:	water
GLP:	In compliance
Study period:	January 2012 - February 2012

HC Yellow No. 17 was tested for mutagenicity in the reverse mutation assay on bacteria in two independent experiments in the absence and presence of metabolic activation (S9-mix prepared from the livers of rats given Aroclor 1254). The experiments were conducted according to the direct plating incorporation method, apart from the second test with S9-mix which was performed according to the pre-incubation method. Toxicity was measured on the basis of a reduction in the number of revertant colonies and/or clearing of the bacterial background lawn. Since HC Yellow No. 17 was freely soluble at 50 mg/ml, concentrations used were based on the cytotoxicity limits. *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537 were exposed to the test substance at concentrations ranging from 5 – 5,000 µg/plate. The negative and positive controls were in accordance with OECD guideline.

Results
In several strains slight toxic effects, evident as a slight thinning of the background lawn and a slight reduction in the number of revertants in the test groups with and without S9-mix, were detected at the higher 2 concentrations. A clear toxic effect evident as a clear thinning of the background lawn and a clear reduction in the number of revertants was detected at the highest concentrations for strains TA98, TA1537 and TA102 without S9-mix and for TA1535, TA1537 and TA102 with S9-mix.

All solvent and positive controls gave counts of revertants within expected ranges, and the experiments were therefore considered to be valid. When compared to controls, no biologically relevant increases in the number of revertants were observed after treatment with HC Yellow No. 17, either in the absence or presence of S9-mix.

Conclusion
Under the conditions of this study, HC Yellow No. 17 was not mutagenic in this gene mutation test in bacteria.

Ref. 7

***In vitro* mammalian cell gene mutation test in mouse lymphoma cells (*hprt* locus)**

Guideline/Method:	OECD 476,
Test system:	Mouse lymphoma L5178Y cells
Replicates:	Duplicate cultures, two independent experiments
Test substance:	HC Yellow No. 17
Batch:	E212289 BCF-52281/5
Purity:	relative purity 95.5 % pure at 428 nm
Solvent	water
Concentrations:	Experiment 1: In the absence of S9-mix: 200, 300, 350, 400, 450, 500, 550, 600 and 650 µg/mL In the presence of S9-mix: 80, 120, 160, 200, 250 and 300 µg/mL Experiment 2: In the absence of S9-mix: 200, 400, 450, 500, 550, 600 and 625 µg/mL In the presence of S9-mix: 50, 100, 150, 200, 250 and 300 µg/mL
Treatment:	3-hour treatment with and without S9-mix; expression period 7 days and a selection period of 11 days
Positive Controls:	without S9-mix: 4-nitroquinoline (NQO), with S9-mix: Benzo[a]pyrene (B[a]P),
Negative control:	purified water
GLP:	In compliance
Study period:	January-June 2012

HC Yellow No. 17 was evaluated for mutagenicity in Mouse lymphoma cell line L5178Y in *hprt* locus for 6-thioguanine (6-TG) resistance in two independent experiments using duplicate cultures each (single cultures for positive controls). Both experiments used 3-hour treatment and were conducted in the absence and presence of metabolic activation (S9-mix) prepared from the liver of rats given Aroclor 1254. Test concentrations were based on the results of a pre-test on toxicity measuring relative survival growth. In the main tests, cells were treated for 3h with and without S9-mix followed by an expression period of 7 days to fix the DNA damage into stable *hprt* mutations. The negative and positive controls were in accordance with OECD guideline.

Results
The range-finding study demonstrated that on the basis of both cytotoxicity and solubility criteria the selected concentrations were between 80 and 650 µg/ml. In experiment 1 with

3h treatment in the absence of S9-mix cytotoxicity was 21% and 9% relative survival at 600 and 650 µg/mL respectively, in the presence of S9-mix and with 24 h treatment 71% relative survival at 300 µg/mL at which post-treatment precipitation was noted. In experiment 2 in the absence of S9 mix cytotoxicity was 11% relative survival at 625 µg/mL and in the presence of S9 mix 93% relative survival at 300 µg/mL at which post-treatment precipitation was noted.

Mutant frequencies in solvent negative controls fell within normal ranges, and treatment with positive controls NQO and B[a]P yielded distinct increases in mutant frequency with at least one concentration. Accordingly, the study was considered to be valid. When tested up to the limit of cytotoxicity or solubility, there were no statistically significant increases in mutant frequency following treatment with HC Yellow No. 17 at any concentration tested in the absence and presence of metabolic activation. A significant linear trend was observed in the presence of S9-mix in Experiment 2. However, as there were no significant increases in mutant frequency at any concentration analysed in this experiment, the effect was not reproduced between experiments and the mutant frequencies were all within the range of the historical controls, this isolated observation was not considered biologically relevant.

Conclusion
Under the conditions of this study, HC Yellow No. 17 was considered not to be mutagenic in the mouse lymphoma assay (*hprt* locus), either in the absence or presence of metabolic activation.

Ref. 8

SCCS comment
Only short treatment (3 h) was used both with and without S9-mix. Water was used as solvent and precipitation occurred already in low concentrations (300 µg/mL and above) in both experiments with metabolic activation. Due to this, recommended cytotoxicity was not achieved in the experiments with S9-mix. The lowest relative survival in maximum concentration used was 93, and 71% respectively.

***In Vitro* Micronucleus Test in Cultured Human Lymphocytes**

Guideline:	Draft OECD 487 (2004)
Species/strain:	Human peripheral blood lymphocytes of two female voluntaries
Replicates:	Duplicate cultures in one experiment; 4 cultures for the solvent control,
Test substance:	HC Yellow No. 17
Solvent:	purified water
Batch:	E212289 BCF-52281/5
Purity:	relative purity 95.5 % pure at 428 nm
Concentrations:	60, 80 and 100 µg/mL in 3h-treatment ± S9-mix 80, 100 and 120 µg/mL in 24h-treatment -S9-mix
Treatment;	Experiment I: 3h treatment, ± S9-mix Experiment II: 24h treatment, – S9-mix
GLP:	In compliance
Study period:	January-June 2012

HC Yellow No. 17 was evaluated in the absence and presence of metabolic activation (S9-mix prepared from the livers of Aroclor 1254-treated rats). Top concentrations were selected based on precipitation of the test item observed at the end of the treatment incubation period or at harvest and not based on cytotoxicity.

Duplicate cultures were treated with each concentration of HC Yellow No. 17 or with known clastogens in the presence (cyclophosphamide, CPA) or absence of S9-mix (mitomycin C,

MMC and vinblastine, VIN). Solvent-treated cultures (purified water, four replicates) were used as negative controls.
Blood cultures were incubated in the presence of the mitogen phytohaemagglutinin (PHA) for 48 hours and then received a 24- or 3-hour treatment in the absence or presence of S9-mix, respectively. Cells were harvested 72 hours after the beginning of incubation. Cytochalasin B was added after the 3-hour treatments or before the 24-hour treatments.
Lymphocyte preparations were stained and examined microscopically for determining the RI and the proportion of micronucleated binucleated (MNBN) cells when selected. Two thousand binucleate cells per concentration (one thousand from each replicate) were analysed blindly.

Results
In the highest concentrations evaluated after the different treatments, precipitation was observed after treatment or at harvest of the cells. Due to this precipitation, the required cytotoxicity was not reached.
When compared to concurrent solvent controls, treatment of cultures with positive controls CPA, MMC and VIN resulted in consistent significant increases in MNBN frequencies, thus validating the sensitivity of the test system and procedure used.

Treatment of cells with HC Yellow No. 17 in the absence and presence of S9-mix resulted in frequencies of MNBN cells, which were similar and not significantly higher than those observed in concurrent vehicle controls for all concentrations analysed under all treatment conditions. There were marginal increases in MNBN cell frequency in single cultures at the lowest and highest concentrations analysed (60 and 100 µg/mL) following a 3-hour treatment in the absence of S9-mix, though these were small and not observed in replicate cultures with no evidence of any concentration-related effect. The MNBN cell frequency of all other treated cultures fell within normal values. Therefore, these sporadic marginal increases were considered of no biological relevance.

Conclusion
Under the conditions of the study, HC Yellow No. 17 did not produce cells with micronuclei in cultured human peripheral blood lymphocytes either in the absence or presence of metabolic activation and was therefore considered to have no clastogenic or aneugenic potential.

Ref. 9

SCCS comment
Precipitation occurred in each experiment, both with and without metabolic activation and in all treatment conditions, and no cytotoxicity was observed in any of concentration considered for micronucleus evaluation.

3.3.6.2 Mutagenicity / Genotoxicity in vivo

3.3.7 Carcinogenicity

3.3.8 Reproductive toxicity

3.3.8.1 Two-generation reproduction toxicity

/

3.3.8.2 Other data on fertility and reproduction toxicity

3.3.8.3 Developmental Toxicity

Guideline:	OECD 414
Species/strain:	HsdHan: WIST rats
Group size:	24
Test substance:	E212289
Batch:	BCF52281-LR1
Purity:	83.5 % w/w, 97.4 Area % (at 428 nm), 96.7 Area % (at 265 nm)
Vehicle:	Corn oil (suspension)
Dose levels:	100, 300 and 1000 mg/kg bw/d
Dose volume:	5 mL/kg bw
Route:	oral
Administration:	gavage
Treatment period:	days 5 – 19 of gestation
GLP:	in compliance
Study period:	11 Jan – 8 Feb 2013

During the mating period, female rats were cohabited with males in a 1:1 ratio. The day of detection of a sperm-positive vaginal smear/vaginal plug was considered as day 0 of gestation. Ninety-six presumed pregnant female rats were assigned to four groups, 24 rats per group. The test item E212289 was suspended in vehicle (corn oil) and administered orally (via gavage), once daily from day 5 to 19 of gestation at the dose levels of 100, 300 and 1000 mg/kg bw/d, respectively. Vehicle control group animals received corn oil alone, and the dosing volume was 5 mL/kg across all groups. The dams were observed daily for clinical signs and mortality; bodyweight and food consumption were regularly recorded. Caesarean section was performed for all rats on day 20 of gestation, and uterine contents were observed. Foetuses were examined for external (all foetuses), and visceral (half of the foetuses) or skeletal (half of the foetuses) alterations.

Results

There was no mortality throughout the study. No treatment-related clinical signs were observed at 100 and 300 mg/kg bw/d. Reddish brown faeces were observed at 1000 mg/kg bw/d, which can be attributed to the colour of the test item, hence considered to be non-adverse in nature. Administration of test item E212289 resulted in reduced maternal bodyweight gain (-18%) and corrected maternal bodyweight gain (-36%) only at the dose level of 1000 mg/kg bw/d, and other maternal and litter parameters were unaffected.

Foetal external, visceral and skeletal variants and anomalies and litter data parameters were overall similar among groups including vehicle control, and no relevant treatment related observations were noted.

Conclusion

On the basis of the results of this study, the No Observed Adverse Effect Levels (NOAELs) for maternal toxicity and foetal toxicity are 300 mg/kg bw/d and 1000 mg/kg bw/d in Wistar rats, respectively, and test item E212289 was considered to have no teratogenic potential.

Ref. 10

SCCS comment

Some observed changes in the incidences of delayed skeletal ossification at 1000 mg/kg bw/d may be due to maternal toxicity.

3.3.9 Toxicokinetics

3.3.9.1 Toxicokinetics in laboratory animals

3.3.9.2 Toxicokinetics in humans

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity / photo-irritation and photosensitisation

3.3.10.2 Photomutagenicity / photoclastogenicity

3.3.11 Human data

3.3.12 Special investigations

3.3.13 Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Absorption through the skin	**A**	**=**	**0.047 µg/cm²**
Skin Area surface	**SAS**	**=**	**580 cm^2**
Dermal absorption per treatment	**SAS x A x 0.001**	**=**	**0.027 mg**
Typical bodyweight of human		**=**	**60 kg**
Systemic exposure dose (SED)	**SAS x A x 0.001/… bw/d**	**=**	**0.00045 mg/kg**
No observed adverse effect level (developmental toxicity study, oral, rat)	**NOAEL**	**=**	**300 mg/kg bw/d**
Bioavailability 10%*		**=**	**30 mg/kg bw/d**
Margin of Safety	**adjusted NOAEL/SED**	**=**	**66000**

* Because of the large molecular weight and the ionic character of the dye, 10% bioavailability is considered.

3.3.14 Discussion

Physico-chemical properties

The ingredient HC Yellow No. 17 is intended to be used in non-oxidative hair colouring products at a maximum concentration of 0.5%.

No information about the reference standard used for the determination of HC Yellow No. 17 was provided except that it was considered to be 85.7% pure. EC Methods were not used for the determination of water solubility and Log Pow.

General toxicity

No acute oral toxicity studies were submitted with HC Yellow No. 17. However, no deaths were observed in the subchronic (13-week) oral toxicity study performed in rats as well as in the developmental toxicity study in rats at dose levels of 100, 300 and 1000 mg/kg/day (Ref. 6 and Ref. 10).

In an oral subchronic toxicity study in rats the NOAEL of HC Yellow No. 17 is derived at 1000 mg/kg bw/d.

In an oral developmental toxicity study in rats the NOAEL for maternal toxicity and foetal toxicity are 300 mg/kg bw/d and 1000 mg/kg bw/d, respectively. No study on reproductive toxicity was provided.

Irritation/sensitisation

Under the conditions of human Episkin test for skin irritation, it can be concluded that undiluted HC Yellow 17 is non-irritant after dermal application.

On the basis of the results obtained in the BCOP study, it can be concluded that HC Yellow No. 17 at 20% (w/w) in physiological saline is not a strong eye irritant. This does, however, not exclude mild or moderate eye irritancy potential. Under the conditions of the study provided, an eye irritation potential of HC Yellow No. 17 at 20% (w/w) in physiological saline cannot be excluded. Considering that the maximum intended concentration of HC Yellow No. 17 in a hair dye product is 0.5% (w/w) and no severe eye irritation potential is observed in the BCOP test for 20% (w/w) HC Yellow No. 17, it can be assumed that eye irritation will be of limited concern.

Under the conditions of the LLNA study, HC Yellow No. 17 is considered as a strong sensitiser capable of inducing delayed contact hypersensitivity. This is a new hair dye. As a potential allergen in humans its capacity to induce allergic contact dermatitis should be evaluated when exposure in consumers will occur.

Dermal absorption

The study of penetration in dermatomed human skin shows that HC Yellow No. 17 present at 0.5% in a typical hair dye formulation penetrated through human dermatomed skin at an extremely slow rate. The amount of HC Yellow No. 17 considered as absorbed was estimated to be at most 0.021 ± 0.013 µg.eq/cm^2 corresponding to 0.019 ± 0.012% of the applied dose.

SCCS considers that the mean plus 2 standard deviations (0.047 µg/cm^2) should be used for MOS calculation because of the short exposure time.

Mutagenicity

The genotoxicity of HC Yellow No. 17 was investigated for the three endpoints of genotoxicity: gene mutations, structural chromosome aberrations and aneuploidy.

HC Yellow No. 17 did not induce gene mutations in bacteria nor in mammalian cells when evaluated at the *hprt* locus. Exposure of human lymphocytes with HC Yellow No. 17 did not result in an increase of micronucleated binucleated cells.

Purified water was used as solvent. Because of the low solubility, test concentrations of the HC Yellow No. 17 were limited by precipitation and not by cytotoxicity. In the micronucleus test, zero cytotoxicity was observed due to precipitation. The solubility of HC Yellow No. 17 could have been maximised by using an organic solvent such as dimethyl sulfoxide (DMSO). However, precipitate would likely also appear if another solvents were used.

Based on the present available tests and results provided, HC Yellow No. 17 can be considered to have no genotoxic potential and additional tests are unnecessary.

Carcinogenicity
No data were submitted.

4. CONCLUSION

(1) In light of the data provided, does the SCCS consider HC Yellow No. 17 (B121) safe when used in non-oxidative hair colouring products at concentrations of up to 0.5%?

The SCCS considers the hair dye HC Yellow No. 17 (B121) safe when used in non-oxidative hair colouring products at concentrations of up to 0.5%, except for its sensitisation potential. HC Yellow No. 17 is considered a strong sensitiser.

(2) Does the SCCS have any further scientific concerns with regard to the use of HC Yellow No. 17 (B121) in cosmetic products?
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5. MINORITY OPINION

6. REFERENCES

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