Scientific Committee on Consumer Safety

SCCS

OPINION
on Acid Yellow 3 – C054
(CAS No. 8004-92-0, EC. No 305-897-5)
Submission II

The SCCS adopted this document
by written procedure on 23 July 2021

ACKNOWLEDGMENTS

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This Opinion has been subject to a commenting period of eight weeks after its initial publication (from 10 may until 5 July 2021). Comments received during this time period are considered by the SCCS. For this Opinion, no change occurred and conclusions remain unchanged from the preliminary version.

1. ABSTRACT

The SCCS concludes the following:

(1) In light of the data provided, does the SCCS consider Acid Yellow 3, safe when used in non-oxidative hair colouring products up to a maximum on-head concentration of 0.5 %?

Based on the data provided in the dossier, the SCCS considers that Acid Yellow 3 is safe when used in non-oxidative hair colouring products at on-head concentrations of up to 0.5%.

(2) Does the SCCS have any further scientific concerns with regard to the use of Acid Yellow 3 in cosmetic products?

A detailed analytical report on the test substance in representative batches and results of the stability tests should be provided to exclude the possibility of the presence of any impurities that may be of concern.

Keywords: SCCS, scientific opinion, Acid Yellow 3, hair dye, Regulation 1223/2009, CAS Number 8004-92-0, EC No 305-897-5

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

The substance with the INCI name 'Acid Yellow 3' (CI 47005) and chemical name '1H-Indene-1,3(2H)-dione, 2-(2-quinolinyl)-, sulfonated, sodium salts' is listed under entry 82 of Annex IV to the Cosmetics Regulation (EC) No. 1223/2009 and therefore it is allowed for use as a colorant in cosmetic products.

Acid Yellow 3 [mixture of the disodium salts of the mono- and disulfonic acids of 2-(2-quinolyl)-1H-indene-1,3(2H)-dione] is intended to be used in non-oxidative hair colouring products up to a maximum on-head concentration of 0.5 %.

In 2004, the Scientific Committee on Cosmetic and Non-food products (SCCNFP) concluded that the use of Acid Yellow 3 as a hair dye ingredient is deemed acceptable provided that the risk evaluation undertaken for the dietary use of this colouring agent has been based upon sound analysis of appropriate studies. In addition, among other remarks, the SCCNFP stated that the identity of the substance was unclear and recommended that a re-evaluation of the Acceptable Daily Intake (ADI) for this food colour should be performed.

Terms of reference

(1) In light of the data provided, does the SCCS consider Acid Yellow 3, safe when used in non-oxidative hair colouring products up to a maximum on-head concentration of 0.5 %?

(2) Does the SCCS have any further scientific concerns with regard to the use of Acid Yellow 3 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

INCI name Acid Yellow 3

3.1.1.2 Chemical names

Mixture of the disodium salts of the mono- and disulfonic acids of 2-(2-quinolyl)-1H-indene-1,3(2H)-dione

EC name: 1H-Indene-1,3(2H)-dione, 2-(2-quinolinyl)-, sulfonated, sodium salts
IUPAC name: disodium 2-(1,3-dioxo-2,3-dihydro-1H-inden-2-yl) quinoline-6,8-disulfonate

3.1.1.3 Trade names and abbreviations

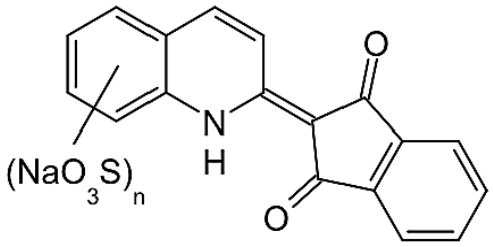
COLIPA n°	C 054
Trade name	D&C Yellow N° 10
Other names	Quinoline Yellow, E104, Food Yellow 13

3.1.1.4 CAS / EC number

CAS:	8004-92-0
EC No:	305-897-5 or 616-849-0*
Colour index:	CI 47005

*ECHA's website for C.I. Food Yellow 13
https://echa.europa.eu/substance-information/-/substanceinfo/100.116.526

3.1.1.5 Structural formula



Ref. 7

3.1.1.6 Empirical formula

$C_{18}H_9NO_8S_2Na_2$
$C_{18}H_{10}NO_5SNa$

3.1.2 Physical form

Yellow powder

3.1.3 Molecular weight

477.41 g/mol ($C_{18}H_9NO_8S_2Na_2$)
375.3 g/mol ($C_{18}H_{10}NO_5SNa$)

3.1.4 Purity, composition and substance codes

Analytical data related to batches AK0828, AK3596 and AK6423 are presented in Table 1.

Purity	
HPLC	89.26% (290 and 418 nm) (qualitative)
Monosulphonated	85-93.7%
Disulphonated	3.6-13.8%
Unsulphonated	> 0.1%
NaCl	0.1-2.8%
Na_2SO_4	0.1-3.5%
Water insoluble matter	max 0.2%
Heavy metals	
Lead	≤ 20 ppm
Arsenic	≤ 3 ppm
Mercury	≤ 1 ppm
Organic compounds other than coloring matters	≤ 0.5 %
2-(2-quinolyl)-1H-indene-1,3-(2H)-dione	≤ 4 ppm

In Table 1, additional information of the batches/lots with FDA certification is provided.

Table1: batches/lots with FDA certification

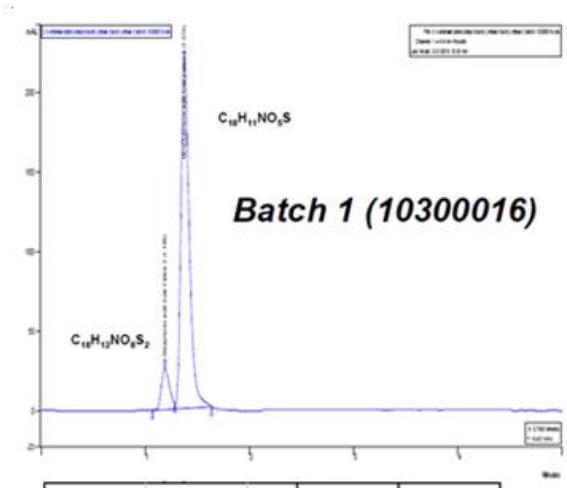
Lot No.	AK0828	AK3596	AK6423
Batch No.	0679AB	DCOYM/1	818AR
FDA Certificate	Yes, 25 March 1999	Yes, 16 November 1999	Yes, 02 August 2000
Total color	90%	87%	91%
Volatile Matter-Max 50	6.3	5.4	5.2
NaCl	1.65	0.1	2.8
Na_2SO_4 by I.C.	0.1	3.5	0.6
Water insoluble Matter	NF*	NF	NF
Intermediates			
Phthalic acid	NF	NF	NF
Sulfonated Quinaldine	0.05	0.1	NF
Quinaldine	NF	NF	NF
Subsidiary Colors			
Monosulfonated	85.0	90.8	93.7
Disulfonated	13.8	3.6	6.9
Unsulfonated	>0.0	>0.1	>0.1
Ether Soluble Matter	NF	NF	NF
Heavy Metals (ppm)			
Mercury	PT#	PT	PT
Lead	PT	PT	PT
Arsenic	PT	PT	PT
NF* =			
PT# =			

Ref. 7

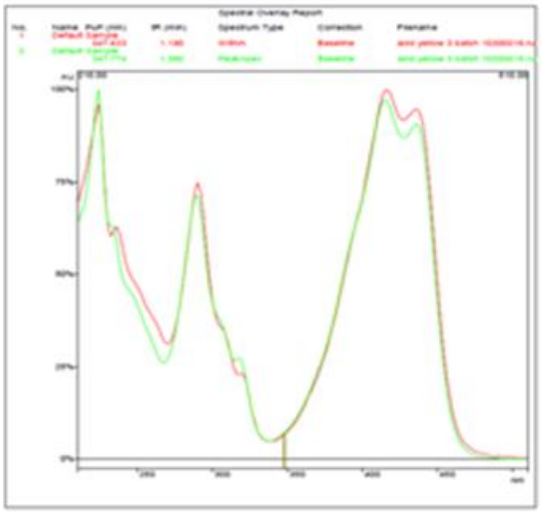
New Submission

Characterisation of the dye Acid Yellow 3 by HPLC & LC MS

Preparation of Control Samples: 100 mg of Acid Yellow 3 were weighted in a 100 ml graduated flask. The flask was filled with 70% acetonitrile. The solution was stirred and sonicated for 10 min. The dye solution was filtered with a nylon filter 0.22 µm into a HPLC vial.



	Rt	Area
Disulphonic Acid Yellow 3	1,18	1390202
Monosulphonic Acid Yellow 3	1,37	12223987



- DAD Spectra Overlay for the dye Acid Yellow 3 in disulfonated and monosulfonated form

In all three dye batches (10300016, 10300017 and 10300018) of Acid Yellow 3, the mono and disulfonated components were determined by HPLC Diode Array Detector (λ: 414 nm) and confirmed by LC-MS analysis. This confirms that the dye Acid Yellow 3 exists in a mono and disulfonated salt form.

Ref. 4

Acid Yellow 3, batch 14070092:

Name:	Acid Yellow 3
Alternative Names:	Dragocolor D&C Yellow N° 10, Quinoline Yellow E104, Food Yellow 13
Batch Number:	14070092
Physical Appearance:	Dark Yellow Powder
Purity:	92.2% (monosulfonic Chinolynylindendione, Na-salt)
Storage Conditions:	Ambient (10°C - 30°C)
Expiration Date:	31 December 2021
Molecular Weight:	375.3 g/mol (monosulfonic Chinolynylindendione, Na-salt) 352.3 g/mol (monosulfonic Chinolynylindendione) 477.0 g/mol (disulfonic Chinolynylindendione, Na salt)

purity: 92.2% (monosulfonic Chinolynylindendione, Na-salt)
Molecular weights:
375.3 g/mol (monosulfonic Chinolynylindendione, Na-salt)
352.3 g/mol (monosulfonic Chinolynylindendione)
477.4 g/mol (disulfonic Chinolynylindendione, Na salt)

Acid Yellow 3, batch 160106255:

Name:	Acid Yellow 3
Alternative Names:	Dragocolor D&C Yellow N° 10, Quinoline Yellow E104, Food Yellow 13
Batch Number:	160106255
Physical Appearance:	Dark Yellow Powder
Purity:	83.54% (monosulfonic Chinolynylindendione, Na-salt)
Storage Conditions:	Ambient (10°C - 30°C)
Expiration Date:	31 October 2023
Molecular Weight:	375.3 g/mol (monosulfonic Chinolynylindendione, Na-salt) 352.3 g/mol (monosulfonic Chinolynylindendione) 477.0 g/mol (disulfonic Chinolynylindendione, Na salt)

purity: 83.54% (monosulfonic Chinolynylindendione, Na-salt)
Molecular weights:
375.3 g/mol (monosulfonic Chinolynylindendione, Na-salt)
352.3 g/mol (monosulfonic Chinolynylindendione)
477.4 g/mol (disulfonic Chinolynylindendione, Na salt)

SCCS comment
Data of the LC-MS studies conducted to confirm the presence of mono and disulfonated salt forms of quinolone yellow were not provided. Purity data of the batches 10300016, 10300017 and 10300018 were not provided. In the batches 14070092 and 160106255 monosulfonic chinolynylindendione (M.W. 352.3 g/mol) was also present in the base form. There is no explanation for PT and NF acronyms in Table 1.

3.1.5 Impurities / accompanying contaminants

Reagents and intermediate reaction products

Sulfonated 2-methyl quinoline	0.05%
2-(2-quinolyl)-1H-indene-1,3-(2H)-dione	< 4 ppm

SCCS comment
A full report on the chemical characterisation of the test substance in terms of impurities in representative batches and the validity of the analytical methodologies used must be provided.

3.1.6 Solubility

20% soluble in water, 5% soluble in saline, 2.5% soluble in DMSO, 2% in standard formulation.

3.1.7 Partition coefficient (Log P_{ow})

Log P_{ow} 0.7 (HPLC method)

3.1.8 Additional physical and chemical specifications

Where relevant:
- organoleptic properties (colour, odour, taste if relevant): no odour
- melting point: >150°C (decomposition)
- boiling point: /
- flash point: /
- vapour pressure: /
- density: /
- viscosity: /
- pKa: /
- pH: /
- refractive index: /
- UV/visible light absorption spectrum: λ_{max} 414 nm

3.1.9 Homogeneity and Stability

Acid Yellow 3 is shown to be stable under conditions used in non-oxidative formulations.

Stability/shelf life:
Stability in the hair formulation: in sponsor's file
Stability in water > 72 hr
Storage: room temperature; light and humidity protected

Ref. 3

SCCS comments
The compound is a mixture of the mono- and disulfonic acid salts of 2-(2-quinolyl)-1H-indene- 1,3(2H)-dione. Two additional peaks are detected by HPLC but these have not been explained.
Details on the stability tests are not provided and should be provided.
Information on the batches is only given for some studies, the majority of which is reported as an overview of the published literature. The actual test results should be provided to the SCCS for assessment of the validity of the data.

3.2 EXPOSURE ASSESSMENT & TOXICOKINETICS

3.2.1 Function and uses

Acid Yellow 3 is used in hair colouring formulations used up to 0.5 % in non-oxidative hair dye formulations. It is common practice for 100 ml of undiluted formulation to be applied for a period of 30 minutes before washing. Due to its very favourable colouring properties, Acid Yellow 3 present in non-oxidative hair colouring product will be applied once per month on-the head.

Acid Yellow 3 is shown to be stable under conditions used in non-oxidative formulations. The application procedure is described as follows:

Type of formulation	Application amount [ml]	Application time [min]	Rinse off	Mixing procedure	Frequency of use
Non-Oxidative	100	30	Yes	No	1/month

3.2.2 Dermal / percutaneous absorption

From a previous submission, the SCCNFP had concluded that the pH of the receptor fluid (3.0) was not appropriate, and therefore the *in vitro* dermal absorption test was not acceptable (SCCNFP/0789/04).

Ref. 6

Additional data provided in submission II

The percutaneous absorption of Acid Yellow 3 (Yellow 10) was investigated with an oxidative hair dye formulation and a non-oxidative hair dye formulation.

However, as Acid Yellow 3 will only be used and marketed in the EU as a non-oxidative hair dye formulation with a content of up to 0.5%, only the study parts related to the testing of the non-oxidative hair dye formulation is presented in the section below.

Guidelines/Guidances:	OECD 428; OECD Guidance document No. 28 SCCP/0970/2006; SCCS/1564/15 COLIPA guidance 1997
Test item:	Acid Yellow 3
batch:	14070092
purity:	92.2% (monosulfonic Chinolynylindendione, Na-salt)
Molecular weights:	375.3 g/mol (monosulfonic Chinolynylindendione, Na-salt) 352.3 g/mol (monosulfonic Chinolynylindendione) 477.4 g/mol (disulfonic Chinolynylindendione, Na salt)
Test item formulation:	Hair dye formulation with 0.5% Acid Yellow 3 for non-oxidative usage
batch:	12.2016 (code: T1 E 2016076 1)
GLP:	yes
Study period:	27 March 2017 – 28 March 2018, report dated 03 September 2018

Test system

Twelve abdominal skin samples of full-thickness human skin were obtained from female and male donors aged 29 to 67 years old. The subcutaneous fat and connective tissue was removed by a scalpel, the skin was washed, dried and cut into pieces and stored at -20ºC. Intermittently, the skin samples were removed from -20°C storage and thawed at ambient temperature. After measuring the thickness of the full-thickness skin pieces using a micrometer, split-thickness membranes were prepared with an electric dermatome (Zimmer®) at a setting equivalent to 350-500 µm depth and controlled again by a micrometer. Thereafter the skin pieces were further processed and stored for a maximum of two months at a temperature of -20 °C prior to use.

For the percutaneous absorption study *in vitro*, the skin pieces were removed from -20 °C storage and thawed carefully at room temperature. Sections of the split-thickness skin

(about 1.5 cm x 1.5 cm) were cut and mounted in the diffusion cells between the donor and receptor chamber.

A static glass diffusion cell system (PermeGear Inc) was used, placed on a magnetic stirrer plate heated via a circulating water bath to maintain the skin surface temperature at 32°C ± 1°C. The surface area of exposed skin within the cells was 0.64 cm² with a nominal receptor chamber volume of 5 mL.

The receptor fluid was phosphate buffered saline, containing sodium azide (*ca* 0.01%, w/v). The pH of the receptor fluid was checked and adjusted to pH 7.33-7.50 squared (rms) in the parallel equivalent circuit mode. Any skin sample exhibiting an electrical resistance of less than 10.9 kΩ was excluded from the subsequent absorption measurements.

A previously developed and validated LC-MS/MS method was used to analyze Acid Yellow 3.

Test procedure

The *in vitro* percutaneous absorption of Acid Yellow 3 was studied by application of the hair dye formulation containing 0.5% Acid Yellow 3 for non-oxidative usage (batch: 12.2016)) evenly over the surface of the *stratum corneum* surface of 12 samples of human split-thickness skin obtained from five donors using a positive displacement pipette set to deliver *ca* 12.8 mg (*ca* 20 mg/cm^2). To accurately quantify the concentration of test preparation applied to the skin samples, seven weighed aliquots (mock doses) of each test preparation were taken at the time of dosing. Methanol (10 mL) was added to all mock dose samples. Samples were placed on a shaker for *ca* 30 min, sonicated for *ca* 30 min and analyzed by LC-MS/MS.

Receptor fluid aliquots were collected at 0.5, 1, 2, 4, 6, 8, 12 and 24 h post dose.

At 30 min post dose, the exposure period was terminated by applying ten washes of water, followed by a single application of sodium dodecyl sulphate solution in water (2%, v/v). The skin was then rinsed with a further ten washes of water and finally dried with three tissue swabs, which were retained as a single sample. Methanol (10 mL) was added to all tip and tissue swab samples.

After a further 23.5 h post exposure monitoring period (*i.e.* at 24 h post dose), the skin samples were washed and sampled as they had been after the 30 min. exposure period.

The donor chamber was transferred to a pot containing methanol. The skin was removed from each cell and placed on a piece of tissue to remove any remaining receptor fluid from the underside of the skin. This tissue was placed into the receptor chamber wash pot for that particular cell.

Thereafter, the *stratum corneum* was removed with 20 successive tape strips (D-Squame tape discs). The tape strips were grouped in glass vials in the sequence of Tape Strips 1-2, Tape Strips 3-5, Tape Strips 6-10, Tape Strips 11-15 and Tape Strips 16-20.

The skin under the cell flange (unexposed skin) was cut away from the exposed skin. The exposed epidermis was then separated from the dermis by heat separation and by using a scalpel. Skin samples and cling film were placed into individual glass vials. Methanol (10 mL) was added to all tape strip, unexposed skin, exposed epidermis, exposed dermis and cling film samples. Samples were placed on a shaker for *ca* 30 min and sonicated for *ca* 30 min prior to analyses.

Donor chambers were extracted in methanol for >30 min before sonication (10 min). Donor chambers were then removed from the pots. The remaining receptor fluid (bulk receptor fluid) was collected in vials and stored in a freezer set to maintain a temperature of -20°C for future reference. The receptor chambers were rinsed with methanol (20 mL)

and the solvent was pooled as a single sample into a receptor wash pot. All samples were stored in a freezer set to maintain a temperature of -20°C before analysis by LC-MS/MS.

Results

For any data less than the LLOQ value of 1 ng/mL, the LLOQ value has been used for results calculation. This results in a worst-case scenario for absorbed dose and dermal delivery.

Acid Yellow 3 was detectable in all of the receptor chamber samples and skin extracts.

A total of 12 samples of human split-thickness skin membranes obtained from five different donors were dosed topically with the hair dye formulation containing 0.5% Acid Yellow 3 for non-oxidative usage. The mass balance for all individual samples, with the exception of Cell 20 (125.49%), was within 100 ± 15%. Therefore, cell 20 was excluded from the statistical analysis and the results are provided as mean values (n = 11).

The mean mass balance was 96.71% of the applied dose at 24 h post dose. The majority of the applied dose was washed off at 30 min post application (75.06%, 3.25% and 0.30% was recovered in the skin wash, tissue swabs and pipette tips, respectively).

At 24 h post dose, a further 4.11% was removed with the wash. A proportion of the dose applied was recovered from the donor chamber (0.24%). Therefore, the total dislodgeable dose was 82.96% of the applied dose. The mean total unabsorbed dose was 91.95% of the applied dose, consisting of the total dislodgeable dose, unexposed skin (0.02%) and *stratum corneum* (8.97%). The amounts retained by the *stratum corneum* at 24 h are not considered to be dermally absorbed.

The absorbed dose (0.04%) was the sum of the receptor fluid (0.01%) and the receptor chamber wash (0.03%) values. The exposed epidermis (including cling film) and exposed dermis contained 4.54% and 0.19% of the applied dose, respectively. Dermal delivery (4.77%) was the sum of the absorbed dose and the exposed epidermis and dermis samples.

At 24 h post dose the mass balance, total dislodgeable dose, unabsorbed dose, absorbed dose and dermal delivery values were 109, 93.1, 103, 0.05 and 5.35 µg/cm², respectively.

Details of the results are summarised in the Table 2.

Table 2:

Actual Acid yellow 3 concentration (%, w/w) 0.564 (112.8 µg per cm²)	**Expressed as µg/cm² of skin surface mean S.D. (n = 11)**			**Expressed as % of substance dose mean S.D. (n = 11)**		
Dislodgeable Dose (30 min)	88.2	±	5.38	78.61	±	4.79
Total Dislodgeable Dose (24 h)	93.09	±	5.45	82.96	±	4.85
Total Unabsorbed Dose	103.18	±	4.05	91.95	±	3.61
Total Absorbed Dose	0.05	±	0.00	0.04	±	0.00
Mass Balance	108.53	±	4.61	96.71	±	4.11
Dermal Delivery (bioavailable portion)	5.35	±	2.76	4.77	±	2.46

Dislodgeable dose 30 min = skin wash 30 min + tissue swab 30 min + pipette tip 30 min. Total dislodgeable dose = dislodgeable dose 30 min + skin wash 24 h + tissue swab 24 h + pipette tip 24 h + donor chamber wash. Total unabsorbed dose = total dislodgeable dose + *stratum corneum* + unexposed skin.
Total absorbed dose = cumulative receptor fluid + receptor chamber wash.
Dermal delivery = absorbed dose + exposed epidermis (containing cling film) + exposed dermis. Mass balance = unabsorbed dose + dermal delivery.

Conclusion

Acid Yellow 3 was shown to have a low skin penetration potential when tested *in vitro* on split-thickness human skin in a static diffusion cell system.

Following the open topical application of the hair dye formulation containing 0.5% Acid Yellow 3 for non- oxidative usage, Acid Yellow 3 was detected in the samples relevant for dermal absorption, i.e. in the skin extracts as well as in the receptor fluid sample after 24 hours.

Thus, Acid Yellow 3 is considered to have penetrated the skin and to have become biologically available. Under the reported conditions, the bioavailable portion (dermal delivery) of Acid Yellow 3 is 5.35 ± 2.76 µg/cm² or 4.77 ± 2.46 % (mean value of 11 diffusion cells (5 donors)). The mass balance for Acid Yellow 3 was at the valid level of 96.71% ± 2.46 %.

Ref. 5

SCCS comments
The data obtained from the dermal absorption study carried out according to the most recent guidelines and guidance requirements under non-oxidative conditions show that Acid Yellow 3 has a low dermal penetration. The absorption into viable skin layers and the receptor fluid was 5.35 + 2.76 = 8.11 µg/cm² (Mean + 1xSD). For safety assessment, the SCCS will use dermal absorption value of 8.11 µg/cm² (Mean + 1x SD).

3.2.3 Other studies on toxicokinetics

/

3.2.4 Calculation of SED/LED

/

3.3 TOXICOLOGICAL EVALUATION

The toxicological data were established between 1960 and 1980. They were evaluated in detail by FDA, JECFA (Joint Expert Committee of FAO and WHO, latest update) and the EU (The acceptable daily intake (ADI) was set at 0-10 mg/kg bw/day).
For use in cosmetics, the compound was evaluated by the German DFG (Farbstoffkommission der Deutsche Forschungsgemeinschaft). A toxicity profile including a summary toxicological data was compiled by BIBRA in 1990.

The Table 3 below gives an overview of the data provided on characterisation and toxicological aspects.

Table 3: data provided on characterisation and toxicological aspects

Study type	Lot No.	Batch No.	FDA certification
N-Octanol/Water partition coefficient	AK6423	818AR	yes 02 Aug 2000
Acute oral toxicity	-#	-	-
Primary skin irritation	-	K-7059 (Kohnstamm)*	-
Primary eye irritation	-	-	-
Sensitisation/Modified Buhler Method	-	K-7059 (Kohnstamm)	-
Sensitisation / Guinea Pig Maximisation Test	-	-	-
Sensitisation/Human Maximisation Test	-	-	-
Skin permeability *in vitro*	AK0828	0679AB	yes 25 Mar 1999
Mutagenicity study *in vitro* / Bacterial Reverse Mutation Test	AK0828	0679AB	yes 25 Mar 1999
Mutagenicity study *in vitro* / Cell Mutation Assay at the Thymidine Kinase Locus (TK +/-) in Mouse Lymphoma L5178Y Cells	AK0828	0679AB	yes 25 Mar 1999
Mutagenicity study in vivo / Micronucleus Test in Mice	AK3596	DCOYM/1	yes 16 Nov 1999
Micronucleus Test in Human Lymphocytes in vitro		10300022 / WE Charge 200301136	Yes AZ1094
Long term toxicity / Long term chronic /carcinogenicity/teratogenicity study	-	-	-

Long term toxicity /In utero, long term rat feeding study (summary of FDA data)	-	-	-
-# = no information available			

Ref. 6, 7

3.3.1. Irritation and corrosivity

3.3.1.1 Skin irritation

The SCCNFP (SCCNFP/0789/04) concluded from a previous submission that the provided information, supplied as two references without detailed information on the study design and quality, had reported Acid Yellow 3 as slightly irritant to the skin.

Ref. 6

3.3.1.2 Mucous membrane irritation / eye irritation

The SCCNFP (SCCNFP/0789/04) concluded from a previous submission that the provided information, supplied as a reference without detailed information available on the study design and quality, had reported Acid Yellow 3 as slightly irritant to the mucous membranes.

Ref. 6

3.3.2 Skin sensitisation

The SCCNFP (SCCNFP/0789/04) concluded from a previous submission that the skin sensitisation potential of acid yellow 3 was assessed in a modified Buehler and a guinea pig maximisation test. No skin reactions were observed in either test method. Similarly, no sensitisation was observed in a human maximisation test with 15 volunteers. Based on these data, acid yellow 3 was not considered to be a skin sensitiser.

Ref. 6

3.3.3 Acute toxicity

3.3.3.1 Acute oral toxicity

LD50 (rat) > 2 g/kg bw
LD50 (rat) > 2 g/kg bw
LD50 (rat) > 5 g/kg bw
LD50 (dog) > 1 g/kg bw

The SCCNFP (SCCNFP/0789/04) from a previous submission concluded that the data in the dossier were taken from the literature or unpublished reports, and as such no study details were provided.

Ref. 6

3.3.3.2 Acute dermal toxicity

No data provided.

3.3.3.3 Acute inhalation toxicity

No data provided.

3.3.4 Repeated dose toxicity

From SCCNFP/0789/04

Summary of toxicological studies

At present, no studies on acute, subacute, subchronic, chronic, reproductive and developmental toxicity and carcinogenicity are available to the SCCNFP for evaluation. From the documents supplied by the applicant, it can be derived that many of such studies were performed between 1960 and 1980. The studies are summarized in Table 4.

The Scientific Committee on Food (SCF) established an ADI value of 0-10 mg/kg bw/day, based on a NOEL of 1000 mg/kg bw/day in a long-term mice study (table 4, study n° 11). The same study was evaluated by JECFA and the same ADI value was estimated. FDA derived an acceptable daily intake of 10 mg/kg bw/day, based on a NOAEL of 1000 mg/kg bw/day, corresponding to 2% in the diet. This was based on 2 chronic toxicity and carcinogenicity feeding studies in rats and mice (Table 4, studies n° 8 and 9). These studies were additionally evaluated by BIBRA and the DFG. Based on body and organ weight changes, BIBRA and DFG deduced a NOAEL of 250 mg/kg bw/day, corresponding to 0.5% in the diet.

Furthermore, it has to be mentioned that the evaluation of a reproductive toxicity study by BIBRA (study 12 in the Table 4) concluded that an increase in postnatal mortality and a decrease in postnatal weight gain occurred at and above 0.5%. From these findings, a NOAEL of 50 mg/kg bw/day could be deduced.

Table 4: an overview of the toxicity studies reviewed in submission I.

No.	study type / Species / No.	Application route / time / doses	Source / evaluation	Parameters / effects	NOEL
1	Subacute / cat / number unknown	Po / 7 d / 100 mg/kg bw	Oettel et al. 1965 / DFG + BIBRA	Red blood cells / no effect	/
2	Subchronic / rat / 5m + 5 f	diet / 90 d / 0, 0.25, 0.5, 1.0, 2.0, 5.0 %	Hansen et al. 1960 / DFG + BIBRA	Body weight, food consumption, haematology, organ weight / No adverse effects	2500 mg/kg bw/d
3	Subchronic / dog / number unknown	diet / 90 d / 3 %	Hazleton 1962 / DFG	Parameters not given/ Body weight reduction	/
4	Subchronic / rat / 20 m + 20 f	diet / 90 d / 3 %	Hazleton 1965 / BIBRA	Body weight, clinical signs, haematology, urinalysis, pathology / no effect	1500 mg/kg bw/d
5	Chronic / rat / 10 m + 10 f	Sc / 7 m / 50 mg/kg bw/d	Oettel et al. 1965 / BIBRA	Parameters not given / no effects	> 50 mg/kg bw/d

6	Chronic / mouse / 60 m + 60 f	diet / 23-24 m max. dose 5 %	Biodynamics 1980 / BIBRA	Parameters not given / no effects	> 7500 mg/kg bw/d
7	Chronic / dog / 3 m + 3 f	diet / 2 y / 0.03, 0.2 %	Hazleton 1967 / BIBRA	Parameters not given / no effects	> 70 mg/kg bw/d
8	Chronic toxicity / carcinogenicity / rat / 70 m + 70 f	diet / 30 m, exposure start in utero / 0.03, 0.1, 0.5, 2.0, 5.0 %	Biodynamics 1980 & 1981 / DFG + BIBRA + FDA	Body weight, clinical signs, ophthalmoscopy, organ weight, pathology / reduction of body and several organ weights without tissue damage at 2 and 5 %, no carcinogenicity	FDA: 1000 mg/kg bw/d BIBRA, DFG: 250 mg/kg bw/d
9	Chronic toxicity, carcinogenicity / mouse/60 m + 60 f	diet / 30 m / 0.03, 0.1, 0.5, 2.0, 5.0 %	Biodynamics 1980 & 1981 / DFG + BIBRA + FDA	Body weight, clinical signs, ophthalmoscopy, organ weight, pathology / no carcinogenic effects	/
10	Chronic toxicity, carcinogenicity, exposure start in utero / mouse / 50 m + 50 f	diet / 21-23 m, exposure start in utero / 0, 0.1, 0.3, 1.0, 3 %	IFREB 1981 / DFG + BIBRA	Body weight, mortality, pathology / reduction white blood cells count high dose, no carcinogenic effects	300-1000 mg/kg bw/d
11	Chronic toxicity, carcinogenicity, Reprotox / mouse / 65 m + 65 f	diet / 9 w prior to mating until (Fo) and 21-23 months (F1) / 0, 0.1, 0.3, 1.0%	IFREB 1981 (Coquet et al.) / BIBRA + JECFA	a) Fertility parameters / no effects b) mortality, body weight, haematology, pathology / no effects	1000 1500 mg/kg bw/d
12	Reprotox / rat / 60 m + 60 f	diet / exposure start 2 m prior to mating until end of lactation / 0, 0.5, 5.0, 15.0, 50 mg/kg bw	Biodynamics 1980 & 1981 / DFG + BIBRA	Mortality (adult, postnatal), fertility, weight gain / postnatal mortality increase and postnatal weight gain decreased at and above 0.5 %	50 mg/kg bw/d
13	3 generations exposure start 2 weeks prior to mating / rat / 10 m + 20 f	Diet / 3 generations / 0, 0.5, 5.0, 15.0, 50 mg/kg bw	Smith 1973 (Biodynamics 1973) / DFG + BIBRA	Mortality, body weight; food consumption, mating, fertility, pathology / no adult toxicity, no Reprotoxicity	> 50 mg/kg bw/d

14	Teratogenicity / rat / 20 per dose	Po / 6-15 day of gestation / max. dose 150 mg/kg bw	Biodynamics 1972 / BIBRA	Parameters not given / no effect	> 150 mg/kg bw/d
15	Teratogenicity rabbit / 15	Po / 6-18 day of gestation / max. dose 150 mg/kg bw	Biodynamics 1972 / BIBRA	Parameters not given / no effect	> 150 mg/kg bw/d

Ref. 6

Submission II dossier

No new repeated dose toxicity data provided in submission II (Ref 7).

SCCS comments
The previously established Acceptable Daily Intake (ADI) of 0-10 mg/kg bw/day by the FAO (1974) was revised following evaluation by the WHO (1975, 1978 and 1984) and the EU Scientific Committee for Food (SCF) (1984) to 0.5 mg/kg bw/day. This was based on the NOAEL of 50 mg/kg bw/day, derived from the chronic toxicity and carcinogenicity study with a reproductive toxicity phase in rats, and applying an uncertainty factor of 100 to this NOAEL.

3.3.4.1 Repeated dose (28 days) oral / dermal / inhalation toxicity

See 3.3.4.3

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

See 3.3.4.3

3.3.4.3 Chronic (> 12 months) toxicity

Chronic toxicity and carcinogenicity study with a reproductive toxicity phase in rats (study 12 in the table 4)

Guidelines/Guidances: No information available
GLP: No information available
Test item: D&C Yellow10
Vehicle: Diet
Experimental animals Species: Rat
Strain: Albino
Sex: Males and females
Animal numbers: 60 males and 60 females per group

Test procedure
A long-term chronic toxicity/carcinogenicity study with a reproductive toxicity phase was carried out in male and female albino rats according to the following scheme:
Long-term study for F0:

Groups of 60 male and 60 female F0 generation rats were each fed certified D&C Yellow10 in their diet for 60 days. The following dose levels were tested in this study: 0, 0.03; 0.1 and 0.5 % (equivalent to 0, 15, 50 and 250 mg/kg bw/day, 1st experiment).

2nd experiment: 0, 2.0 and 5.0 % (equivalent to 0, 1000 and 2500 mg/kg bw/day) were administered. Then animals were mated on a 1:1 ratio for one week and F1 litters were delivered.
Criteria evaluated for F0 generation: mortality, general behaviour, detailed physical examination, periodic body weight, periodic food consumption.

Lifetime study phase (F1 generation, pups of F0 dams)
F1 pups were separated by sex one week post weaning and randomly selected for in utero, long-term feeding study. The study is designed to evaluate the effect of D&C Acid Yellow 3 when administered to F1 albino male and female rats 60/sex/group at dietary levels of 0, 0.03, 0.1 and 0.5% for 24 months.
Viability at birth, mean pup weight and sex were determined. Data were also obtained for day 4, 14 and 21 of lactation

Results
Result for F0 generation: No adverse effects were noted for the F0 generation.
Result for F1 generation (pups of F0 dams): Reduced viability and lower weight gains during lactation at dose levels of 0.5 % in the diet (equivalent to 250 mg/kg bw/day) and above. No other treatment-related effects on reproductive parameters were noted.

Conclusion
In the long-term chronic toxicity/carcinogenicity study with a reproductive toxicity phase in male and female albino rats receiving 0, 0.03, 0.1, 0.5, 2, or 5 % D&C Yellow10 in the diet (equivalent to 0, 15, 50, 250, 1000 or 2500 mg/kg bw/day), reduced viability and lower weight gains in pups derived from F0 dams during lactation at dose levels of 250, 1000 and 2500 mg/kg bw/day were observed. These effects were considered as indicative of a treatment-related effect. Thus, the NOAEL under the conditions of this study was 50 mg/kg bw/day.

Ref. 7

SCCS comments
The SCCS agrees with the former evaluations performed which defined a NOAEL of 50 mg/kg bw / day obtained in the chronic toxicity and carcinogenicity study with a reproductive toxicity phase in rats.

3.3.5 Reproductive toxicity

3.3.5.1 Fertility and reproduction toxicity

See 3.3.4.3

3.3.5.2 Developmental Toxicity

See 3.3.4.3

3.3.6 Mutagenicity / genotoxicity

3.3.6.1 Mutagenicity / genotoxicity *in vitro*

SCCS comments on Genotoxicity/Mutagenicity Submission I
The SCCNFP (SCCNFP/0789/04) concluded from a previous submission that Acid Yellow 3 had been tested in a bacterial reverse mutation assay and in a mammalian cell gene mutation assay *in vitro*: the test item was considered non-mutagenic and non-clastogenic in mammalian and bacterial cells.
Acid Yellow 3 was also tested *in vivo* in mice for the induction of micronuclei in the bone marrow cells; there was no demonstration that the substance had reached the target cells and therefore the study was considered inadequate for use in safety assessment.

Ref. 6

Submission II
The applicant concluded that single oral application of Acid Yellow 3, when tested up to the limit dose of 2000 mg/kg bw to male and female mice and indicating bioavailability due to the noted clinical findings, failed to show a significant induction of micronuclei. Thus, Acid Yellow 3 was demonstrated to be non-mutagenic/clastogenic in this murine micronucleus study.

Ref. 7

SCCS conclusion from submission II
Based on the data available, the SCCS concluded that:
Acid Yellow 3 has been tested in a bacterial reverse mutation assay and in a mammalian cell gene mutation assay *in vitro* with negative results. It has also been tested *in vivo* in mice for the induction of micronuclei in the bone marrow cells (Honarvar N., Micronucleus assay in bone marrow cells of the mouse with D&C Yellow 10, RCC-CCR Test report, No. 741301, Rossdorf/Germany, 2003). However considering that:

- there were no signs of toxicity to the bone marrow cells after the exposure to Acid Yellow 3, and more importantly
- the measurements of Acid Yellow 3 (page 34 of the report) indicated that its concentration in serum was below detection level, it is very likely that the test item did not reach the target tissue. Hence, the study was considered inadequate for use in safety assessment.

The SCCS was of the opinion that no convincing evidence was provided to exclude the potential of Acid Yellow 3 to induce chromosomal aberrations. Therefore, the Applicant was requested to provide a valid test on chromosomal aberrations.

In response to the SCCS request, the Applicant provided the following additional *in vitro* study on micronucleus test in human lymphocytes.

***In vitro* Micronucleus Test in human lymphocytes**

Guideline:	OECD 487 (adopted 29 July 2016)
Species/strain:	Cultured human peripheral blood lymphocytes from one male volunteer
Replicates:	Duplicate cultures, 3 experiments
Test substance:	Acid Yellow 3 / Dragocolor D&C Yellow No. 10 / Cl 47005
CAS No.:	8004-92-0 84864-68-6

95193-83-2
FDA Certificate LOT No.: AZ1094
Molecular weight: 477.41 g/mol ($C_{18}H_9NO_8S_2Na_2$)
375.3 g/mol ($C_{18}H_{10}NNaO_5S$)
Batch: 10300022 / WE Charge 200301136, FDA Lot. AZ 1094
Purity: 94.5%

Concentrations: Preliminary test (range-finder):
±S9 mix (4 h exposure): 13.7, 24.1, 42.1, 73.7, 129, 226, 395, **691, 1209, 2116** µg/mL

Exp2:
+S9 mix (4 h exposure): 226, 395, **691, 1209, 2116** µg/mL

Exp3:
-S9 mix (20 h exposure): 129, 226, 395, **691, 1209, 2116** µg/mL
Evaluated experimental points are shown in **bold characters**

Solvent/negative control: concurrent solvent controls (culture medium with 10.0% deionised water
local tap water deionised at ICCR-Roßdorf GmbH)
culture medium
Positive Controls: -S9 mix: MMC; mitomycin C (pulse treatment)
Purity: 98 % dissolved in deionised water
concentration: 0.8 µg/mL

Demecolcine (continuous treatment)
Purity: ≥ 98 % dissolved in deionised water
Concentration: 150 ng/mL

+S9 mix:
Name: CPA; cyclophosphamide
Purity: 97.0 – 103.0% dissolved in saline (0.9 % NaCl [w/v])
Concentration: 20.0 µg/mL

Metabolic activation phenobarbital +ß- Naphthoflavone induced rat liver homogenate (S9)
Vehicle: deionised water
GLP: In compliance
Study period: July 2020 – February 2021

Material and methods
Stock formulations of the test item and serial dilutions were made in deionised water. The final concentration of deionised water in the culture medium was 10 %. The solvent was chosen due to its solubility properties and its relative non-toxicity to the cell cultures.
All formulations were prepared freshly before treatment and used within two hours of preparation. The formulation is assumed to be stable for this period unless specified otherwise by the Sponsor.
Blood samples were drawn from healthy non-smoking donors not receiving medication. For this study, blood was collected from a male donor (19 years old) for Experiment I and III and from a female donor (20 years old) for Experiment II. The lymphocytes of the respective donors have been shown to respond well to stimulation of proliferation with PHA and to positive control substances.

Human lymphocytes were stimulated for proliferation by the addition of the mitogen PHA to the culture medium for a period of 48 hours. The time from start of exposure to cell harvest

was approximately 2 – 2.5 x AGT (average generation time). Any specific cell cycle time delay induced by the test item was not accounted for directly.

Culture conditions
Blood cultures were established by preparing an 11 % mixture of whole blood in medium within 30 hrs after blood collection. The medium was supplemented with penicillin/streptomycin (100 U/mL/100 µg/mL), the mitogen PHA (3 µg/mL as solvent lyophilizate), 10 % FBS (foetal bovine serum), 10 mM HEPES and the anticoagulant heparin (125 U.S.P.-U/mL).
Phenobarbital/β-naphthoflavone induced rat liver S9 was used as the metabolic activation system.

With regard to the molecular weight and the purity (94.5%) of the test item, 2116 µg/mL were applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations ranging from 13.7 to 2116 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity. In the pre-test for toxicity, no precipitation of the test item was observed at the end of treatment. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.

The experimental part with S9 mix was repeated with the same top dose (Exp. II) due to statistically significant increases in micronucleated cells.
No cytotoxic effects were observed in Experiment I and II after 4 hours treatment in the absence and presence of S9 mix. Therefore, 2116 µg/mL were chosen as top treatment concentration for the experimental part without S9 mix and continuous treatment (Experiment III).

Pre-experiment
A preliminary cytotoxicity test was performed to determine the concentrations to be used in the main experiment. Cytotoxicity is characterised by the percentages of reduction in the CBPI in comparison to the controls (% cytostasis) by counting 500 cells per culture. The experimental conditions in this pre-experimental phase were identical to those required and described below for the mutagenicity assay.
The pre-test was performed with 10 concentrations of the test item separated by no more than a factor of √10 and a solvent and positive control. All cell cultures were set up in duplicate.
Exposure time was 4 hrs (with and without S9 mix). The preparation interval was 40 hrs after start of the exposure.
This preliminary test was designated Experiment I, since the cultures fulfilled the acceptability criteria and appropriate concentrations could be selected for cytogenetic evaluation.

Cytogenetic Experiment
Pulse exposure (4 hr exposure)
About 48 hrs after seeding, 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL culture medium was added. After 4 hrs the cells were spun down by gentle centrifugation for 5 minutes. The cells were re-suspended in complete culture medium with 10 % FBS (v/v) and cultured for a 16-hour recovery period. After this period Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.

Continuous exposure (20 hrs exposure, without S9 mix)
About 48 hrs after seeding, 2 blood cultures (10 mL each) were set up in parallel in 25 cm² cell culture flasks for each test item concentration. The culture medium was replaced with complete medium (with 10 % FBS) containing the test item. After 20 hours the cells were spun down by gentle centrifugation for 5 minutes. After washing, the cells were re-

suspended in complete culture medium containing 10 % FBS (v/v). Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.

Preparation of cells
The cultures were harvested by centrifugation 40 hrs after the beginning of treatment, and the cells were stained with Giemsa. In each experimental group, two parallel cultures were analysed. At least 1000 binucleate cells per culture were scored for cytogenetic damage on coded slides. To determine a cytotoxic effect, the CBPI was determined in 500 cells per culture and cytotoxicity is described as % cytostasis.

Results
Three independent experiments were performed. In Experiment I, the exposure periods were 4 hours with and without S9 mix. In Experiment II, the exposure period was 4 hours with S9 mix. In Experiment III the exposure period was 20 hours without S9 mix.
The highest treatment concentration in this study, 2116 µg/mL was chosen with regard to the molecular weight (>200 g/mol) and the purity (94.5%) of the test item and with respect to the OECD Guideline 487 for the *in vitro* mammalian cell micronucleus test.
In this study, no precipitation of the test item in the culture medium was observed at the end of treatment.
No relevant influence on osmolarity or pH was observed.

In all experimental parts in the absence and presence of S9 mix, no cytotoxicity was observed up to the highest applied concentration.
In Experiment I and III in the absence of S9 mix, no relevant increases in the numbers of micronucleated cells were observed after treatment with the test item.
In the presence of S9 mix, statistically significant increases in micronucleated cells were observed after treatment with 691 and 1209 µg/mL (0.68 and 1.30 %). The higher value exceeded the 95% control limit (0.00 – 1.03 % micronucleated cells) and the min-max range (0.05 – 1.25 % micronucleated cells) of the historical solvent control data, but no dose-dependency, tested by trend test, was observed.
In the confirmatory Experiment II in the presence of S9 mix, no relevant increases in the numbers of micronucleated cells were observed after treatment with the test item. The mean percentage of the micronuclei in all treatment groups was within the 95% historical control limits. None of the values were statistically significantly increased when compared to the solvent control and no dose-dependency, tested by trend test, was observed. Therefore, the observations in Experiment I in the presence of S9 mix can be regarded as biologically irrelevant.
Demecolcine (150 ng/mL), MMC (0.8 µg/mL) and CPA (20.0 µg/mL) were used as positive controls and showed distinct increases in cells with micronuclei.

Conclusion
In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce micronuclei as determined by the *in vitro* micronucleus test in human lymphocytes.
Therefore, Acid Yellow 3 / Dragocolor D&C Yellow No. 10 / Cl 47005 is considered to be non-mutagenic in this *in vitro* micronucleus test, when tested up to the highest required concentration.

Ref. 8

SCCS comment
The Applicant provided a valid study. The results, however, indicate there was no dose-dependency, although statistically significant increases in micronucleated cells were observed in the presence of S9 mix after treatment with 691 and 1209 µg/mL (0.68 and 1.30 %), and the higher value exceeded the 95% control limit (0.00 – 1.03 % micronucleated cells) and the min-max range (0.05 – 1.25 % micronucleated cells) of the historical solvent control data.

Also, in the confirmatory Experiment II, no relevant increases in the numbers of micronucleated cells were observed after treatment with the test item in the presence of S9 mix. The substances used as positive controls showed significant induction of MN frequency in the test cells. Therefore, the observations in Experiment I in the presence of S9 mix can be regarded as biologically irrelevant.
The SCCS is of the opinion that Acid Yellow 3 / Dragocolor D&C Yellow No. 10 / Cl 47005 is not genotoxic in the micronucleus test.

Overall SCCS comments on mutagenicity / genotoxicity
Acid Yellow 3 tested in a valid bacterial reverse mutation assay and in a mammalian cell gene mutation assay *in vitro* showed negative results. It has been tested *in vivo* in mice for the induction of micronuclei in the bone marrow cells with inconclusive results. The Applicant has also provided a valid micronucleus assay *in vitro* on human lymphocytes with negative result.

Based on the overall experimental evidence, the SCCS is of the opinion that Acid Yellow 3 can be considered as having no genotoxic potential.

3.3.7 Carcinogenicity

The SCCNFP (SCCNFP/0789/04) concluded from a previous submission that no data on carcinogenicity had been published following the evaluation made by the FDA, JECFA and the SCF that would have led to a change in the evaluations.

Ref. 6

3.3.8 Photo-induced toxicity

3.3.8.1 Phototoxicity / photo-irritation and photosensitisation

No data provided

3.3.8.2 Photomutagenicity / photoclastogenicity

No data provided

3.3.9 Human data

/

3.3.10 Special investigations

/

3.4 SAFETY EVALUATION (including calculation of the MoS)

The previously established Acceptable Daily Intake (ADI) of 0-10 mg/kg bw/day by the FAO (1974) was revised following evaluation by WHO (1975, 1978 and 1984) and the EU Scientific Committee for Food (SCF) (1984) to 0.5 mg/kg bw/day. This was based on the NOAEL of 50 mg/kg bw/day obtained in the chronic toxicity and carcinogenicity study with a reproductive toxicity phase in rats and applying an uncertainty factor of 100 to the NOAEL.

Based on this NOAEL as the Point of Departure (POD), the SCCS has revised the exposure calculation for non-oxidative conditions in accordance with the changed procedure for SED calculations (SCCS/1628/21).

The dermal absorption data from studies carried out according to most recent guidelines and guidance requirements under non-oxidative conditions, showed a low penetration into the viable skin layers and into the receptor fluid (5.35 + 2.76). The SCCS has used the dermal absorption value of 8.11 µg/cm² (Mean + 1x SD) in Margin of Safety (MoS) calculations as below:

CALCULATION OF THE MARGIN OF SAFETY

Absorption through the skin	**DA_a = 8.11 µg/cm²**
Skin Surface area of scalp exposed	**SSA = 580 cm²**
Dermal Absorption per treatment	**SSA x DA_a x 0.001 = 4.7 mg**
Typical body weight of human	**= 60 kg**
Systemic exposure dose (SED)	**SSA x DA_a x 0.001/bw = 0.0783 mg/kg bw**
No observed adverse effect level	**NOAEL = 50 mg/kg bw/d**
(oral chronic toxicity and carcinogenicity study in the rat with a reproductive toxicity phase, rat)	
Bioavailability 50%*	**$NOAEL_{sys}$ = 25 mg/kg bw/d**

Margin of Safety:	
adjusted $NOAEL_{sys}$/SED	**= 25 mg/kg bw /d / 0.0783 mg /kg bw/ d**
-> MoS: 319	

* standard procedure according to the SCCS's Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation.

3.5 DISCUSSION

Physicochemical properties

The compound is a mixture of the mono- and disulfonic acid salts of 2-(2-quinolyl)-1H-indene- 1,3(2H)-dione. A full report on the chemical characterization of the test substance in terms of impurities in representative batches must be provided and the validity of the analytical methodologies used must be shown. Details on stability tests are not provided and should be provided.

Exposure assessment & Toxicokinetics

Dermal absorption
The data obtained from the dermal absorption study carried out according to most recent guidelines and guidance requirements under non-oxidative conditions show that Acid Yellow 3 has a low dermal penetration. The absorption into viable skin layers and the receptor fluid was 5.35 + 2.76 µg/cm². For safety assessment, the SCCS will use a dermal absorption value of 8.11 µg/cm² (Mean + 1x SD).

Function and uses
Acid Yellow 3 is intended to be used as a non-oxidative hair dye formulation in a concentration of up to a maximum content of 0.5% to be applied for a period of 30 min once per month.

Toxicological Evaluation

Irritation and corrosivity

Based on a previous submission, the SCCNFP Opinion (SCCNFP/0789/04) has noted that Acid Yellow 3 is slightly irritant to both the skin and the mucous membranes.

Skin sensitisation

In a previous submission to the SCCNFP (SCCNFP/0789/04), the skin sensitisation potential of Acid Yellow 3 was assessed in a modified Buehler and a Buehler and a guinea pig maximisation test. No skin reactions were observed in both test methods. In a human maximisation test with 15 volunteers, no sensitisation was observed. Based on these data, Acid Yellow 3 was not considered to be a skin sensitiser.

Acute toxicity

The acute toxicity of Acid Yellow 3 can be considered as very low. The acute oral LD50 has been reported as >2000 mg/kg bw in rat and >1000 mg/kg bw in dog.

Repeated dose toxicity

A long-term chronic chronic/carcinogenicity study with a reproductive toxicity phase was conducted in albino rats with Acid Yellow 3 at 0, 2.0 and 5.0 % (equivalent to 0, 1000 and 2500 mg/kg bw/day) for 24 months. No adverse effects were noted in the F0 generation.

Reduced viability and lower weight gains during lactation had been registered at 0.5 % in the diet (equivalent to 250 mg/kg bw/day) and above. Since no other treatment-related effects were noted, a NOAEL of 50 mg/kg bw/day was derived from this study.

Reproductive toxicity

See above

Mutagenicity / genotoxicity

Acid Yellow 3 tested in a valid bacterial reverse mutation assay and in a mammalian cell gene mutation assay *in vitro* showed negative results. It has been tested *in vivo* in mice for the induction of micronuclei in the bone marrow cells with inconclusive results. The Applicant has also provided a valid micronucleus assay *in vitro* on human lymphocytes with negative result.
Based on the overall experimental evidence, the SCCS is of the opinion that Acid Yellow 3 can be considered as having no genotoxic potential.

Carcinogenicity

See 3.3.4.3

Photo-induced toxicity

No data provided.

Human data

No data provided.

4. CONCLUSION

(1) In light of the data provided, does the SCCS consider Acid Yellow 3, safe when used in non-oxidative hair colouring products up to a maximum on-head concentration of 0.5 %?

Based on the data provided in the dossier, the SCCS considers that Acid Yellow 3 is safe when used in non-oxidative hair colouring products at on-head concentrations of up to 0.5%.

(2) Does the SCCS have any further scientific concerns with regard to the use of Acid Yellow 3 in cosmetic products?

A detailed analytical report on the test substance in representative batches and results of the stability tests should be provided to exclude the possibility of the presence of any impurities that may be of concern.

5. MINORITY OPINION

/

References related to dossier on Acid Yellow 3

1. EFSA (2009) Scientific Opinion on the re-evaluation of Quinoline Yellow (E 104) as a food additive, EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2009; 7(11):1329

2. EFSA (2013) Scientific Opinion on the safety and efficacy of Quinoline Yellow (E104) as a feed additive for non-food-producing animals, EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), European Food Safety Authority (EFSA), Parma, Italy, EFSA Journal 2013; 11(7):3320

3. KAO Europe (2003) COLIPA No. C 054, INCI: Yellow 10, HAIR DYE, Mixture of the sodium salts of the mono- and disulfonic acids of 2(2-quinolinyl)-1H-indene-1,3(2H)-dione, SUBMISSION I, September 2003

4. KAO Europe (2018) Characterization of the dye Acid Yellow 3 by HPLC & LC-MS, KAO Salon Division, Germany, December 2018

5. Kerin T (2018) The *In Vitro* Percutaneous Absorption of Acid Yellow 3 in Two Hair Dye Formulations Through Human Skin, Charles River Laboratories Edinburgh Ltd, Tranent, UK, Study Number 799629, 03 September 2018

6. SCCNFP (2004) Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products intended for consumers concerning Acid Yellow 3, COLIPA No C54, SCCNFP/0789/04, adopted by the SCCNFP during the 28th plenary meeting of 25 May 2004

7. CoE. SUBMISSION II (ADDENDUM TO SUBMISSION I, SEPTEMBER 2003) January 10th 2020

8. CoE. SUBMISSION III (ADDENDUM TO SUBMISSION II, October 10th 2018). Naumann S. 2021 Acid Yellow 3/Dragocolor D&C Yellow No. 10 / Cl 47005: Micronucleus Test in Human Lymphocytes *In vitro*. ICCR Study Number: 2121000-Roßdorf GmbH

7. GLOSSARY OF TERMS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181

8. LIST OF ABBREVIATIONS

See SCCS/1628/21, 11th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – from page 181