Scientific Committee on Consumer Safety

SCCS

SCIENTIFIC ADVICE ON HC Yellow No. 16 (Colipa No. B123)

(CAS No. 1184721-10-5)

Submission II

The SCCS adopted this document by written procedure on

on 31 July 2024

ACKNOWLEDGMENTS

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SCCS

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Scientific Committees (europa.eu)

1. ABSTRACT

The SCCS concludes the following:

1. *In light of the data provided, does the SCCS consider HC Yellow 16 safe, when used as an ingredient at 1 % in oxidative hair dye and at 1.5 % in non-oxidative hair dye formulations?*

In the light of the data provided, SCCS considers that the use of HC Yellow No. 16 (B123) as an ingredient at 1% in oxidative hair dye formulations and at 1.5% in non-oxidative hair dye formulations is safe, considering the specifications of the new commercial batches as described in the submission II file.

2. *Does the SCCS have any further scientific concerns with regard to the use of HC Yellow 16 in cosmetic products?*

/

Keywords: SCCS, scientific advice, hair dye, HC Yellow No. 16, B123, Submission II, CAS 1184721-10-5, Regulation 1223/2009

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), scientific advice on hair dye HC Yellow No. 16 (Colipa No B123) (CAS No. 1184721-10-5) – Submission II, preliminary version of 31 July 2024, SCCS/1670/24

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

The ingredient with the INCI name 'HC Yellow 16' and chemical name '2-chloro-4-[(1E)-(1-methyl-1H-pyrazol-5-yl)diazenyl]-phenol' (CAS No. 1184721-10-5, EC No. -) may be used as an ingredient in oxidative and non-oxidative hair dye formulations.

In 2015, the Commission services received a dossier from industry to support the safe use of 'HC Yellow 16' in cosmetic products. In its corresponding opinion (SCCS/1568/15)[1], the SCCS concluded that '*…the use of HC Yellow No. 16 (B123) as an ingredient at 1 % in oxidative hair dye formulations and at 1.5 % in non-oxidative hair dye formulations is safe*'. In addition, the SCCS stated that '*The purity of HC Yellow No. 16 and impurities in it are not adequately quantified. Data on purity and impurities of HC Yellow No. 16 (B123) should be provided, together with purity specifications of the substance intended for use in cosmetic products*".

With the current submission (i.e., submission II), received in February 2024, the applicant requests to assess the safety of 'HC Yellow 16' in view of the newly provided information on its purity, when used as an ingredient at 1 % in oxidative hair dye formulations and at 1.5 % in non-oxidative hair dye formulations.

Terms of reference

1. In light of the data provided, does the SCCS consider HC Yellow 16 safe, when used as an ingredient at 1 % in oxidative hair dye and at 1.5 % in non-oxidative hair dye formulations?

2. Does the SCCS have any further scientific concerns with regard to the use of HC Yellow 16 in cosmetic products?

[1] https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_192.pdf

3. SCIENTIFIC ADVICE

From previous Opinion (SCCS/1568/15)

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1. Primary name and/or INCI name

HC Yellow No. 16 (INCI)

3.1.1.2 Chemical names

2-chloro-4-[(1E)-(1-methyl-1H-pyrazol-5-yl)diazenyl]-phenol

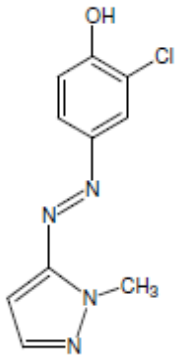
3.1.1.3 Trade names and abbreviations

T44P2

3.1.1.4 CAS / EC number

CAS: 1184721-10-5
EC: /

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

$C_{10}H_9ClN_4O$

3.1.2 Physical form

Yellow powder

3.1.3 Molecular weight

Molecular weight: 236.7 g/mol

3.1.4 Purity, composition and substance codes

Additional data provided in submission II

According to the Applicant, the already high purity of HC Yellow No. 16 could be further improved by the manufacturer. This reflects in a further reduction in concentration and/or number of impurities in the current commercial batches of HC Yellow 16 compared to the batches (FF#20080829, T-9609-9610, 20.05.08, 11-001) used in the SCCS Opinion SCCS/1568/15. As these old batches do not represent the current commercial quality of the dye anymore and only the quality of the current commercial quality will be available on the market, the Applicant submitted the requested data by using three current commercial batches.

Table 1. Overview of the batches used in original document submitted to the SCCS ("Old Batches") and the batches used for providing the requested data ("New Commercial Batches")

Old Batches	New Commercial Batches
FF#20080829	Y16_18005_181 206 314
T-9609-9610	Y16_18006_181 206 350
20.05.08	Y16_18007_190 220 122
11-001	

The sample 20.05.08 was not available anymore for analysis. No data will be shown for this old Batch.

New market specification

Table2. Purity

Overall Purity (HPLC)	greater than 99%
Water Content	< 1%
Impurity 5B	≤ 0.124%
Impurity 7B	≤ 0.026%
Impurity 8B	≤ 0.212%
Heavy Metal Content*	Arsenic < 5 ppm Antimony < 5 ppm Lead < 5 ppm Cadmium < 0,5 ppm Mercury < 0,3 ppm Iron < 20 ppm
Remaining solvent	Methanol < 3000 ppm Acetone < 5000 ppm Triethylamine < 5000 ppm

*As, Pb, Cd and Fe were measured by ICP-MS after submission II

^{1}H and ^{13}C NMR were obtained for the three HC Yellow 16 new commercial batches. The proton of the OH-group did not result in a sharp signal which indicates a proton-deuterium exchange. The solvent signals were used as reference (^{1}H: δH 2.500 ppm residual DMSO-d5 in DMSO-d6, ^{13}C: δC 39.56 ppm).
All 7 protons and all 9 carbon atoms could be detected in the NMR spectra.

Y16_18005_181 206 314

^{1}H NMR (500 MHz, DMSO) δ 11.23, 7.91, 7.91, 7.80, 7.80, 7.79, 7.78, 7.58, 7.57, 7.16, 7.15, 6.49, 6.48, 4.14, 2.50, 2.50, 2.50, 2.49.
^{13}C NMR (126 MHz, DMSO) δ 156.71, 152.42, 145.64, 138.91, 125.13, 122.89, 121.19, 116.82, 93.28, 35.95.

Y16_18006_181 206 350

^{1}H NMR (500 MHz, DMSO) δ 11.22 (s, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.7, 2.4 Hz, 1H), 7.58 (d, J = 2.2 Hz, 1H), 7.16 (d, J = 8.7 Hz, 1H), 6.48 (d, J = 2.2 Hz, 1H), 4.15 (d, J = 2.5 Hz, 3H).
^{13}C NMR (126 MHz, DMSO) δ 156.70, 152.41, 145.64, 138.90, 125.12, 122.89, 121.19, 116.82, 93.28, 35.94.

Y16_18007_190 220 122

^{1}H NMR (500 MHz, DMSO) δ 11.22 (s, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.79 (dd, J = 8.7, 2.4 Hz, 1H), 7.58 (d, J = 2.2 Hz, 1H), 7.16 (d, J = 8.7 Hz, 1H), 6.48 (d, J = 2.2 Hz, 1H), 4.14 (s, 3H).
^{13}C NMR (126 MHz, DMSO) δ 156.71, 152.42, 145.64, 138.90, 125.13, 122.89, 121.19, 116.82, 93.28, 35.94.

All ^{1}H and ^{13}C NMR spectra show the expected quantity of proton and carbon signals in DMSO-d6. The results of all three batches are comparable in terms of chemical shift, number of protons/carbon atoms and spin coupling.

IR spectra of HC Yellow 16 were measured in solid form on a Bruker Alpha II FT-IR spectrometer in the frequency range of 4000 to 400 cm^{-1} at room temperature (22 °C). All FT-IR spectra of HC Yellow 16 showed the same absorption bands in the for each compound unique fingerprint area between 1600 and 400 nm^{-1}.

The heavy metal content of the three commercial batches of HC Yellow 16 was determined by inductively coupled plasma mass spectrometry (ICP-MS) following DIN EN ISO 17294-2. Arsenic, lead, cadmium, mercury and iron were below the limit of quantification.

SCCS comment

In the ^{1}H NMR spectrum of the sample Y16_18005_181 206 314, the signals appearing at 2.50 ppm and 2.49 ppm correspond to the solvent DMSO-d6.

3.1.5 Impurities / accompanying contaminants

Additional data provided in submission II

Data for water and heavy metal content, remaining solvent, and residue on ignition were taken from the specification sheet delivered for each batch by the dye manufacturer.

Table 3. Data available in the specification sheet of the new commercial batches.

Impurity	Specification	Y16_18005 181 206 314	Y16_18006 181 206 350	Y16_18007 190 220 122
Total heavy metal	20 ppm or less	20 ppm or less	20 ppm or less	20 ppm or less
Iron	50 ppm or less	20 ppm or less	20 ppm or less	20 ppm or less
Arsenic	2 ppm or less	2 ppm or less	2 ppm or less	2 ppm or less
Remainig solvent Methanol	3000 ppm or less	493 ppm	449 ppm	557 ppm
Remaining solvent Aceton	5000 ppm or less	404 ppm	266 ppm	244 ppm
Remaining solvent Triethylamine	5000 ppm or less	188 ppm	171 ppm	96 ppm
Water content	1.0% or less	0.05%	0.07%	0.06%
Residue on ignition	1.0% or less	0.02%	0.03%	0.02%

The water content was determined using the Karl-Fischer method.

The TTC approach was used (Appendix I) for the determination of the safety levels of the remaining solvents (methanol, aceton and thiethylamine). As an overarching worst-case assessment for all contained solvents, the Applicant used Cramer Class III (90 µg/person/day) and the highest specified solvent concentration (5000 ppm) for their calculations (Table 4).
According to the Applicant, no risk must be expected from each contained individual solvent in HC Yellow 16 at the on-head use concentrations of 1.5% under non-oxidative or 1.0% under oxidative conditions.

Table 4. Overarching worst-case risk assessment for the three contained solvents contained in HC Yellow 16 using the TTC approach

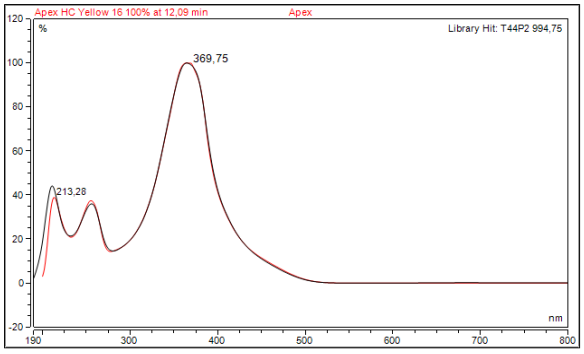
Product type	SED [µg/person/day]	TTC [µg/person/day]	Margin of Exposure
Individual solvent			
Non-oxidative hair dye formulation	18.77	90	5
Oxidative hair dye formulation	8.25	90	11

SCCS comment
The SCCS considers the TTC approach followed by the Applicant to be scientifically acceptable for justifying the safety of the remaining solvents present at levels < 5000 ppm in the final product. Although it is conservative enough, the SCCS notes that the TTC value (90 µg/person/day) does not follow the SCCS Note of Guidance (SCCS/1647/22). The currently recommended value by the SCCS for cosmetics-related substances lacking genotoxic alert classified in Cramer class III is 138 µg/person/day.

Rational of using new HC Yellow 16 Batches
To ensure consistency of the data in respect to the old batches, the Applicant provided additional HPLC data to demonstrate the improvement in quality for the new commercial batches.

Peak purity was determined by comparing the found UV-Vis spectrum at different retention times within the peak of the HC Yellow 16 peak.
The LOD for HC Yellow 16 with the aforementioned HPLC method was determined to be below 5.7 ppb (= 0,00000057 %).
Purity and impurities of HC Yellow 16 were determined for all old and new commercial batches, using the same method and HPLC machine at both 254 nm and 371 nm (371 nm = absorption maximum of anionic/deprotonated HC Yellow 16 with the HPLC solvent system).

Figure 1. Absorption spectrum of HC Yellow 16 as received from the HPLC measurement. Comparison of measured absorption [red] and data from library [black].



In the UV-Vis spectra obtained by the analysis of the main peak in the HPLC spectrum at 12.09 min (=HC Yellow 16), a single absorption maximum at about 370 nm can be found.

Table 5. Summary of the relative Area in % obtained from the HPLC data of HC Yellow 16 at 254 nm

At 254 nm	FF#20080829	T-9609-9610	11-001	Y16_18005	Y16_18006	Y16_18007
Impurity 1A 9.95			0.121			
Impurity 2A 9.72			0.052			
Impurity 3A 10.49		0.113				
Impurity 4A 10.51 - 10.52	0.049	0.105	0.299			
Impurity 5A 10.66		0.023				
Impurity 6A 11.28 – 11.32	0.083					
HC Yellow 16 12.07 - 12.09	98.906	99.121	98.128	99.799	99.779	99.804
Impurity 7A 13.71-13.74	0.081	0.359	0.103	0.056	0.048	0.051
Impurity 8A 14.58 - 14.59	0.221	0.063				
Impurity 9A 14.77 -14.83	0.200	0.137	0.202	0.145	0.172	0.145
Impurity 10A 15.35		0.078				
Impurity 11A 20.74	0.461					
Impurity 12A 23.47			1.094			
Total	100%	100%	100%	100%	100%	100%

According to the Applicant, purity for the old batches was found to be already high with 98.1+% within three old batches at 254 nm. For the new commercial batches, purity could be even further improved to 99.8+%. Where in the old batches small amounts of 12 different impurities could be found at 254 nm, only two impurities could be detected for the new commercial batches, using the same method at 254 nm (Table 4).

Table 6. Summary of the relative Area in % obtained from the HPLC data of HC Yellow 16 at 371 nm

At 371 nm	FF#20080829	T-9609-9610	11-001	Y16_18005	Y16_18006	Y16_18007
Impurity 1B 10.35	0.013					
Impurity 2B 10.51-10.52	0.086	0.246	0.382			
Impurity 3B 11.13	0.016	0.014	0.024			
Impurity 4B 11.32	0.015	0.007				
HC Yellow 16 12.07-12.09	98.691	98.551	99.018	99.638	99.674	99.671
Impurity 5B 13.72-13.74	0.180	0.736	0.247	0.124	0.121	0.116
Impurity 6B 14.19	0.009					
Impurity 7B 14.58-14.60	0.0141	0.035		0.026	0.016	0.013
Impurity 8B 14.78-14.83	0.256	0.191	0.330	0.212	0.189	0.201
Impurity 9B 15.35-15.41	0.021	0.067				
Impurity 10B 16.40		0.026				
Impurity 11B 17.96	0.034					
Impurity 12B 20.75-20.77	0.538	0.090				
Total	100%	100%	100%	100%	100%	100%

According to the Applicant, for the results obtained at 371 nm, the already high purity of the old batches of 98.6+% could be further increased for the commercial batches to 99.6+%. Additionally, the number of impurities could be reduced from 12 to three in the new commercial batches. The found impurities in the commercial batches at ~13.7 min (Impurity 5B), at ~14.6 min (Impurity 7B), and at ~14.8 min (Impurity 8B) are also present in the old batches (Table 5).

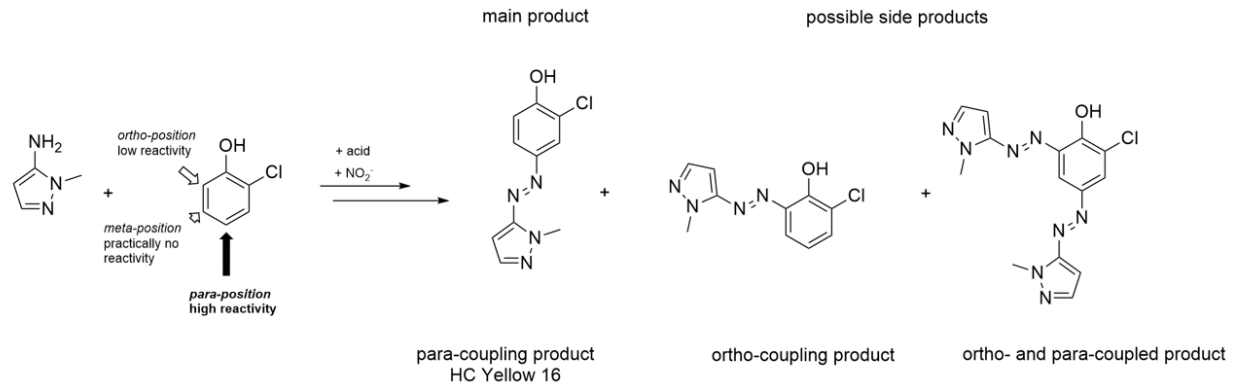
The impurities Impurity 5B (= Impurity 7A), Impurity 7B, and Impurity 8B (=Impurity 9A), found in the new commercial batches for both 254 and 371 nm, are also present in the old batches. In all cases, the maximum concentration of these impurities in the new commercial batches are below the concentrations of the same impurities found in the old commercial batches. The Applicant therefore concludes that the toxicological data conducted with the old batches are also valid for the new commercial batches.

Impurity Characterization for Commercial Batches of HC Yellow 16

Impurity analysis of new commercial batches was performed with HPLC and LC-MS measurements.

As all three impurities show an absorption in the visible range, the Applicant concludes that a chromophoric system must be present in their chemical structure. As the educts of the reaction are color less, the formation of a azo-dye chromophoric system as being present in HC Yellow 16 is the most likely explanation given by the Applicant.

Figure 2. Schematic depiction of azo-coupling reaction including known side products.



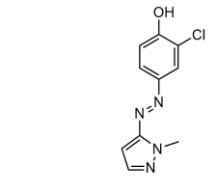
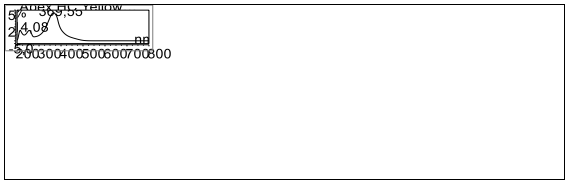
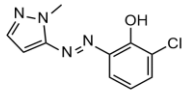
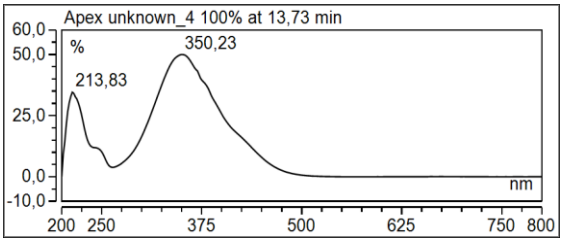
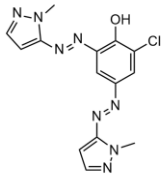
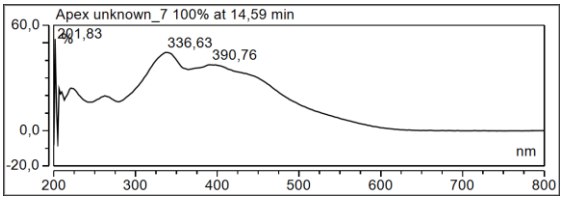
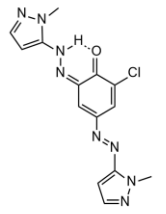
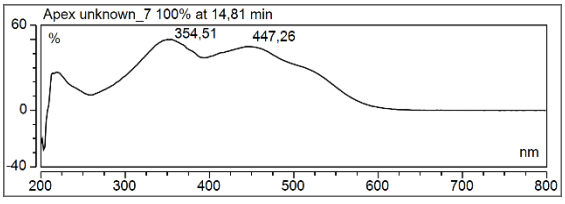
The main reaction product of this azo-coupling reaction is the para-coupling product = HC Yellow 16. Also possible, but due to sterical hindrance far less likely, is the azo-coupling reaction in the ortho-position of ortho-chlorophenol. Depending on the reaction conditions, it is also possible that both ortho- and para-position react, leading to the double-azo coupled dye with a higher molar mass compared to HC Yellow 16. This dye has two independent chromophoric systems (ortho-azo dye and para-azo-dye) in one molecule, leading to two different absoption maxima in the spectrum; one for each chromophore.

To evaluate the final number of different impurities in HC Yellow 16, the UV-Vis spectra as well as the retention time were compared. Based on this comparison, the applicant concluded that the HC Yellow 16 contains three different, coloured impurities.
Impurity 5B at 371 nm = Impurity 7A at 254 nm
Impurity 7B at 371 nm = not found at 254 nm (Sensitivity too low at 254 nm to be detected)
Impurity 8B at 371 nm = Impurity 9A at 254 nm

Representative, batch Y16_18005_181 206 314 was analysed with LC-MS, using a method that was adapted from the HPLC-anylsis and further optimized for LC-MS measurement. Based on the HPLC results, a detection wavelength 371 nm was chosen, as all three impurities are detectable.

All available data for the characterization of the three found impurities in HC Yellow 16 (Impurity 5B, Impurity 7B and Impurity 8B) are summarized in Table 7.

Table 7. Summary of the obtained MS and UV-Vis data by HPLC and LC-MS measurements and the suggested chemical structure

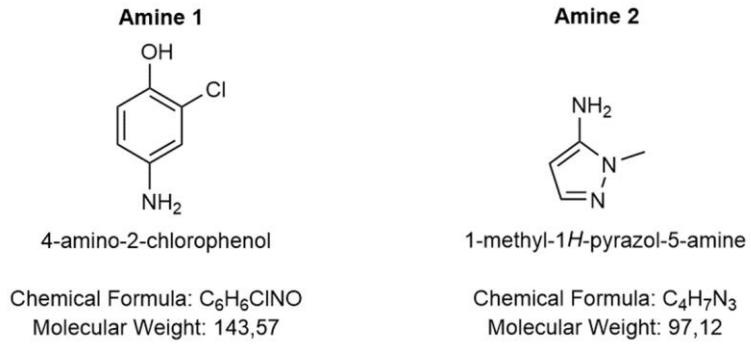
Batch/ retention time HPLC	**MS Data** [M-1]$^-$		**UV-Vis** (representative spectrum)
HC Yellow 16 ~ 12.1 min	235	 Chemical Formula: $C_{10}H_9ClN_4O$ Molecular Weight: 236,66 m/z: 236.05 (100.0%), 238.04 (32.0%), 237.05 (11.0%), 239.05 (3.5%), 237.04 (1.5%) HC Yellow 16	
Impurity 5B ~ 13.7 min	235	 Chemical Formula: $C_{10}H_9ClN_4O$ Molecular Weight: 236,66 m/z: 236.05 (100.0%), 238.04 (32.0%), 237.05 (11.0%), 239.05 (3.5%), 237.04 (1.5%) 2-chloro-6-((1-methyl-1H-pyrazol-5-yl)diazenyl) Phenol	
Impurity 7B ~14.6 min	343	 Chemical Formula: $C_{14}H_{13}ClN_8O$ Molecular Weight: 344,76 m/z: 344.09 (100.0%), 346.09 (32.6%), 345.09 (18.1%), 347.09 (4.9%), 346.10 (1.1%) 2-chloro-4,6-bis((E)-(1-methyl-1H-pyrazol-5-yl)diazenyl)phenol	
Impurity 8B ~14.8 min	343	 Chemical Formula: $C_{14}H_{13}ClN_8O$ Molecular Weight: 344,76 m/z: 344.09 (100.0%), 346.09 (32.6%), 345.09 (18.1%), 347.09 (4.9%), 346.10 (1.1%)	

		No name available	

With UV-Vis and mass analysis, the applicant confirmed the main peak in the HPLC with a retention time 12.1 to be HC Yellow 16. Impurity 5B with a retention time of 13.7 min, was related to the ortho-coupled azo dye. Impurity 7B and Impurity 8B are the ortho- and para-coupled azo reaction product, with Impurity 7B being the azo form and Impurity 8B being the hydrazone form. Both the ortho- and bi-coupled reaction product are known to be side reaction during the favored coupling reaction in para-position (reaction to HC Yellow 16).

The applicant selected two aromatic amines that could potentially be present in HC Yellow 16, based on the sysnthesis (1-methyl-1*H*-pyrazol-5-amine = Amine 2) and degradation of the dye (4-amino-2-chlorophenol = Amine 1).

Figure 3. Chemical structures of the two amines that could potentially be present in HC Yellow 16



They were analyzed using the same HPLC method as for the analysis of HC Yellow 16. According to the Applicant, it was shown, that the method is suitable to detect the amine as low as 2 ppm. Upon comparing the obtained amine HPLC spectra with the HPLC spectrum of HC Yellow 16, no traces of these two amines could be detected.

SCCS comments on purity and impurities - submission II

In the submission II file, the name of the third remaining solvent was not given. Upon request from the SCCS, the Applicant defined the remaining solvent as triethylamine and used the TTC approach to determine the safety levels of the remaining solvents. The SCCS agrees with the Applicant that the concentrations of all remaining solvents (methanol, aceton and thiethylamine) are within the acceptable safe levels.

Upon request from the SCCS, the Applicant provided analytical data on the heavy metal content of the three commercial batches of HC Yellow 16, determined by ICP-MS following DIN EN ISO 17294-2. Arsenic, lead, cadmium, mercury and iron were below the limit of quantification.

The SCCS agrees that the purity of the new commercial batches has been improved. At 371 nm, three impurities were found: Impurity 5B (=Impurity 7A), Impurity 7B and Impurity 8B (=Impurity 9A). At 254 nm, two impurities were found: Impurity 7A and Impurity 9A. These

impurities were also present in the old batches and no new impurity has been detected in the new batches.

3.1.6 Solubility

From previous Opinion (SCCS/1568/15)
Water: 0.5% (pH 9)
DMSO: > 10%
Ethanol: < 1%
Additional data provided in submission II

Solubility of HC Yellow 16 for conducting toxicological studies was determined individually as a part of the respective study.
Generally, the phenolic form of HC Yellow 16 is practically insolube in pure water and shows a medium to good solubility in standard organic solvents like MeOH, EtOH, isoPrOH, DMSO, DMF.
The phenolic dye form was found to be practically insoluble in acetic acid 0.3 %(v/v) and triethylamine 0.3 %(v/v) in H2O (HPLC Solvent B).
The more polar anionic of HC Yellow 16 shows good solubility in water above pH 9.

SCCS comment
The method for water solubility determination is unclear because the study report of water solubility determination was not submitted. Water solubility should be determined by EC Method A.8.

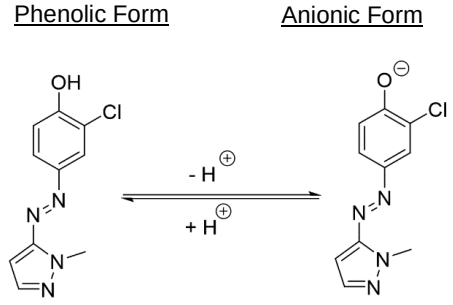
3.1.7 Partition coefficient (Log Pow)

Additional data provided in submission II

Log P_{ow}	3.10 (phenolic form)	Calculated
	0.49 (anionic form)	Calculated

The logarithm of Partition Coefficient ($logP_{ow}$) was estimated using software "molinspiration". This program was chosen as it can predict $logP_{ow}$ data of both phenolic and deprotonated, anionic dye form.

Figure 4. Protonated (phenolic) and deprotonated (anionic) form of HC Yellow 16



This Method for logP prediction developed at Molinspiration (miLogP2.2 - November 2005) is based on group contributions. These have been obtained by fitting calculated logP with experimental logP for a training set more than twelve thousand, mostly drug-like molecules. In this way hydrophobicity values for 35 small simple "basic" fragments have been obtained, as well as values for 185 larger fragments, characterizing intramolecular hydrogen

bonding contribution to logP and charge interactions. No standard deviation is given, according to the applicant.

SCCS comment

Log P_{ow} was not determined by EC Method A.8. The Applicant provided calculated Log P_{ow} values for both the phenolic and the deprotonated, anionic dye form by using a specific software.

3.1.8 Additional physical and chemical specifications

Additional data provided in submission II

Melting point: 231-232 °C (heating table microscope with two heating cycles)
Boiling point: /
Flash point: /
Vapour pressure: /
Density: /
Viscosity: /
pKa: 6.70 (in 2.5% iso-PrOH in water)
Refractive index: /
pH: 6.55-6.57 (suspension of HC Yellow 16 in deionized water at 21 °C)

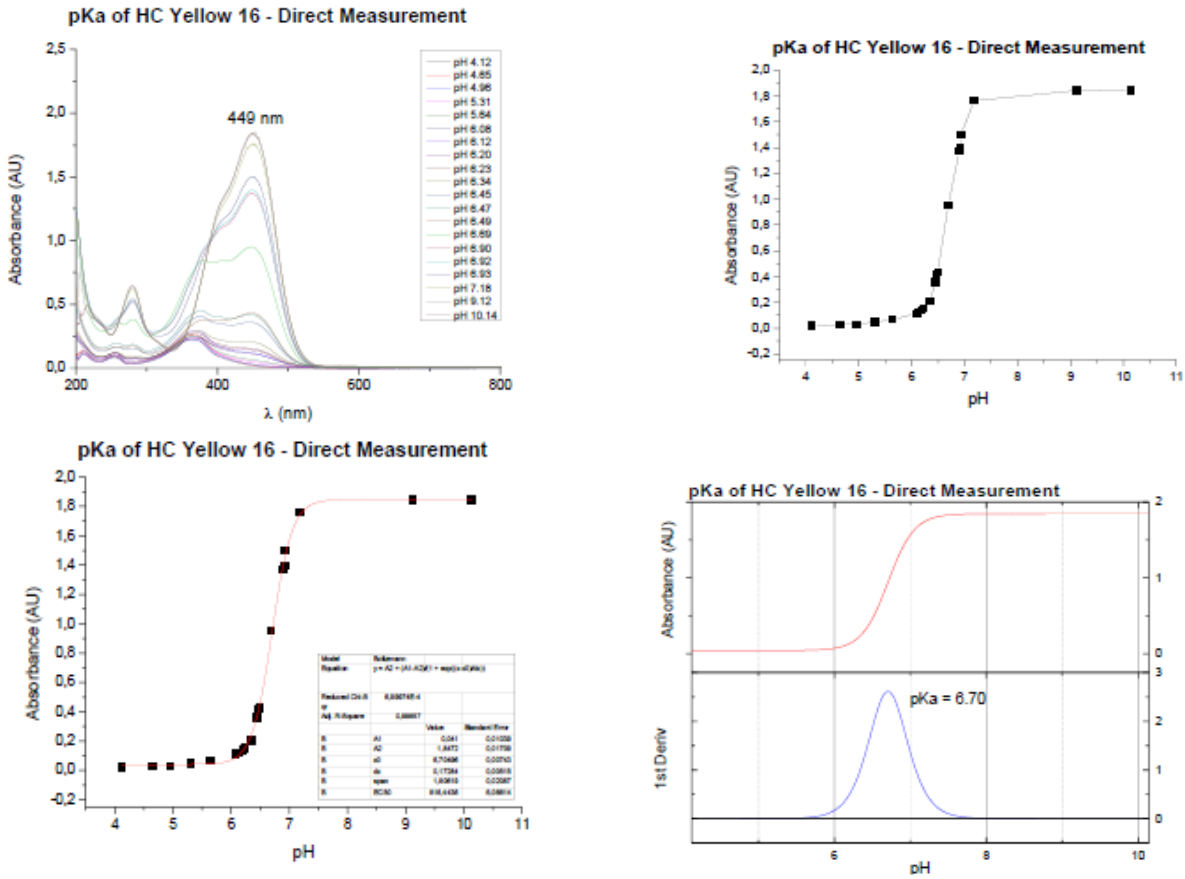
Melting Points of HC Yellow 16 were determined for each batch. Melting points were determined for two heating cycles and summarized in Table 8. The crystalline needles had a sharp melting point between 231 and 232 °C in the second heating cycle.

Table 8. Summary of the melting points of the three HC Yellow 16 samples

	Melting Point [°C]	
	Heating Cycle 1	**Heating Cycle 2**
Y16_18005_181 206 314	232-233	231-232
Y16_18006_181 206 350	231-232	231-232
Y16_18007_190 220 122	229-231	232

Method description for pKa determination: 0.004g (0.02%) of HC Yellow 16 were suspended in 200 ml of iso-PrOH, stirred at 40°C for 1 hr and put into an ultra-sonic bath for 5 min. The solution was allowed to cool down to room temperature. 0.5 g of this dye stock solution was mixed with 19.5 g of buffer solution.
To achieve buffered pH solutions over a range from pH 4.5 to 11.0, the following buffers were used: pH 4.5 to 5.5 = sodium acetate/acetic acid; pH 5.8 to 8.0 = K2HPO4/KH2PO4; pH 8.5 to 11.0 = Na2CO3/NaHCO3
After about 4 hrs, the pH of the sample was determined, and the sample measured using an Agilent Technologies Cary 8454 UV-Vis with an Agilent Peristaltic Pump.

Figure 5. Display of the measured spectra in dependency of the pH [up, left]. At the maximum absorbance at 449 nm, the absorbance was related to the corresponding pH [up, right]. A sigmoidal Boltzmann function was fitted to the data points [down, left] and the maximum of the first derivation is equal to the pKa value of HC Yellow 16 [down, right].

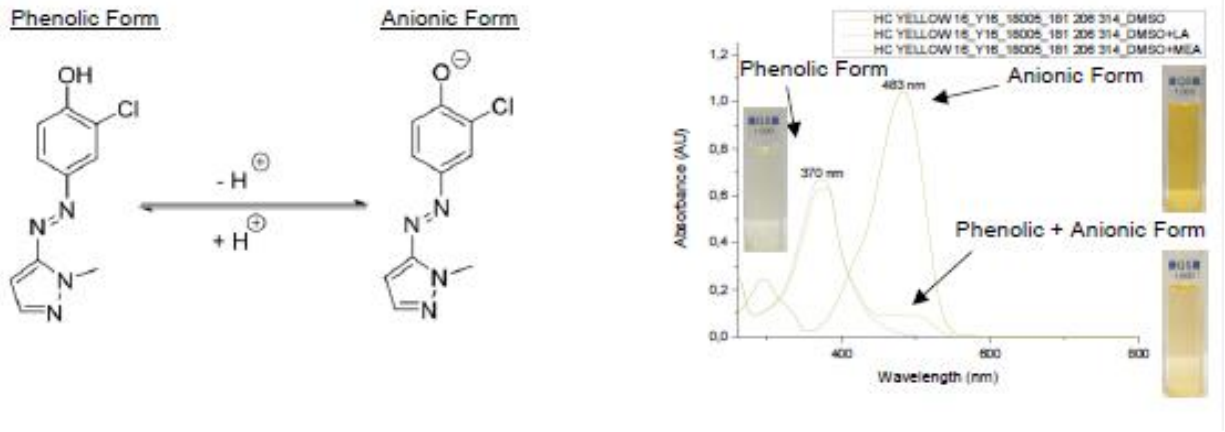


Method description for pH determination in water: 0.5 g of the dye were suspended in deionized water with intense stirring. The suspension was heated in a water bath for 24 hrs at 40°C while stirring to ensure saturation. After 24 hrs, the suspension was allowed to cool down to room temperature (22 °C) for two days and the pH was measured (freshly calibrated Metrohm 913 pH meter).

UV-Vis Spectra of HC Yellow 16

Depending on the pH of the solvent, the anionic, phenolic or a mixture of both forms might be present in the UV-Vis spectra of HC Yellow 16.
In order to demonstrate the pH/solvent dependency of the absorption maximum of HC Yellow 16, each sample was measured using
a) The unaltered, pure solvent
b) The solvent with addition of an acid (acetic acid or lactic acid; fully protonated form)
c) The solvent with addition of a base (MEA or TEA; fully deprotonated form)

Figure 6. Anionic and phenolic form of HC Yellow 16 (left). In pure DMSO (orange spectrum), both the anionic and phenolic form are present in the UVVis spectra (right)



HC Yellow 16 is solvatochromatic, which means that the UV-Vis spectrum is sensitive to the solvent in which it is measured. Depending on the polarity of the solvent, the ground and exited state of the dye are changed, leading to different energy gaps between these two stages. Depending on the solvent, the absorption maximum of HC Yellow 16 will either shift hypso- or bathochromic.
The absorbance maxima for HC Yellow 16 in selected solvents are summarized in Table 9.

Table 9. Summary of solvents used for UV-Vis measurements of HC Yellow 16. A shift in absorption maxima is visible in dependence from the solvent

Solvent		**Absorption Maximum [nm]**	
		Anionic Form	**Phenolic Form**
HPLC Solvents	0.3% (v/v) TEA and 0.3% (v/v) AcOH in MeOH (Solvent A)	362	449
	5% (v/v) iso-PrOH in water	367	457
	HPLC 50%(v/v) n-PrOH, 32% (v/v) AcCN, 8% (v/v) MeOH, 10% (v/v) H_2O	365	460
	DMSO	370	483

In its violet-blue coloured anionic form, HC Yellow 16 shows an absorption maximum between 565 to 619 nm, depending on the solvent. In its protonated, phenolic form, the dye shows a (pale) yellow colour.

Method description: A stock solution of HC Yellow 16 in the given solvents were freshly prepared prior to measurement. As the phenolic form of HC Yellow 16 is practically insoluble in pure water, 5% iso-PrOH was added. Additionally, the dye suspension was shaken at 40°C for 1 hr to accelerate dye dissolution. The suspension was filtered and the obtained stock solution used for measurements.
The stock solution was diluted with pure solvent until an absorption between 0.5 and 2.5 AU was achieved. To 3.2 ml of this diluted dye solution either 0.2 ml of solvent (spectrum of dye in pure solvent), 0.2 ml of acetic acid/lactic acid (spectrum of phenolic form of dye), or 0.2 ml of triethylamine/MEA (spectrum of anionic form of dye) were added and measured using a Agilent Technologies Cary 8454 UV-Vis with a Agilent Peristaltic Pump.

According to the Applicant, the protonation and deprotonation process is reversible and does not influence the chemical stability of the dye. The UV-Vis spectra of HC Yellow 16 is

dependent from the level of deprotonation and type of solvent. Additionally, the concentration of the dye in the solvent can also influence the spectrum as for higher concentrations may lead to interactions between the dye molecules.
The UV-Vis spectrum of HC Yellow 16 in DMSO shows a double peak due to the presence of protonated and deprotonated dye in the solvent. In the other used solvents for UV-Vis measurement, the deprotonated anionic form is dominant prior to pH adjustment.

3.1.9 Homogeneity and Stability

Additional data provided in submission II

Alkaline peroxide stability of HC Yellow 16 was determined using HPLC and UV-Vis. The stability was determined using the batch Y16_18005_181 206 314 for both tests.

Stability of HC Yellow 16 in alkaline peroxide with HPLC
Alkaline peroxide stability of HC Yellow 16 over 45 min at room temperature (22°C) was determined in a buffered alkaline solution (MEA buffer at pH 10). The sample was analysed directly after mixing with 6% peroxide solution (t=0) and after 45 minutes at room temperature (t=45 min). As Reference, the dye solution was mixed with water instead of 6% peroxide solution. The data was evaluated for both 254 and 371 nm.

Table 10. Alkaline peroxide stability of HC Yellow 16 over 45 min in MEA buffer at pH 10 determined by HPLC at 254 and 371 nm (effective dye concentration 0.0115%)

at 254 nm	**R_t in min**	**Area in mA*U**	
Y16_18005 w.6% H2O2 t=0	12.09	41.661	100.00%
Y16_18005 w.6% H2O2 t=45 min	12.09	41.348	99.25% (-0.75%)
Y16_18005 Reference t=0	12.09	41.346	100.00%
Y16_18005 Reference t=45 min	12.09	41.385	100.09 (+0.09%)
at 371 nm	**R_t in min**	**Area in mA*U**	
Y16_18005 w.6% H2O2 t=0	12.09	111.097	100.00%
Y16_18005 w.6% H2O2 t=45 min	12.09	110.897	99.82% (-0.18%)
Y16_18005 Reference t=0	12.07	109.923	100.00%
Y16_18005 Reference t=45 min	12.08	109.998	100.07% (+0.07%)

The stability of HC Yellow 16 in presence of alkaline peroxide over 45 minutes determined via HPLC, was found to be excellent with 99.25% for 254 nm and 99.82% for 371 nm. No new peaks were found in the HPLC data after 45 minutes for 254 and 371 nm under these conditions. According to the HPLC data, HC Yellow 16 can be considered stable under alkaline peroxide conditions.
The provided peroxide stability data in submission II were given with 0.0115% effective dye concentration on the column which reflects all diluting steps upon sample preparation.

The Applicant provided further data with information of the peroxide stability using 0.025% effective concentration.

Method description for 0,025% effective dye concentration: A solution of 0.5% HC Yellow 16 in buffer solution was prepared (4.5% MEA, 5.0% iso-PrOH in water; pH adjusted to 10 with HCl). 4 g of the freshly prepared dye solution was mixed with 4 g of a 6% H2O2 solution in water (ratio = 1:1 w/w) The mixture was stirred intensely for 20 sec. A 1 ml sample of this solution was taken and diluted with 10 ml of water, filtered and the t=0 was measured using HPLC System Dionex Ultimate 3000/ P680 HPLC Pump. After 45 min of intense stirring at room temperature, a 1 ml sample was taken again from this solution measured, diluted with 10 ml of water and measured (t=45 min).

UV-Vis Baseline (blanc)*:* Pure buffer solution without dye was mixed with 6% H_2O_2 solution and measured as background for t=0 and t=45 min.
Reference: To demonstrate that the dye is stable over 45 min in the MEA buffer at pH 10, a 0.5% HC Yellow 16 solution in buffer (see details for buffer above) was prepared and mixed 1:1 w/w with water. After t=0 and after t=45 min upon intense stirring, a 1 ml sample was taken and diluted with 10 ml water and filtered prior to injection to the HPLC.

Table 11. Alkaline peroxide stability of HC Yellow 16 over 45 min in MEA buffer at pH 10 determined by HPLC at 254 and 371 nm (effective dye concentration 0.025%)

at 254 nm	**R_t in min**	**Area in mA*U**	
Y16_18005 w.6% H2O2 t=0	11.85	178.244	100.00%
Y16_18005 w.6% H2O2 t=45 min	11.84	178.220	99.99% (-0.01%)
Y16_18005 Referenz t=0	11.83	176.578	100.00%
Y16_18005 Referenz t=45 min	11.84	176.582	100.06 (+0.06%)
at 371 nm	**R_t in min**	**Area in mA*U**	
Y16_18005 w.6% H2O2 t=0	11.84	542.607	100.00%
Y16_18005 w.6% H2O2 t=45 min	11.83	542.526	99.99% (-0.01%)
Y16_18005 Reference t=0	11.82	546.464	100.00%
Y16_18005 Reference t=45 min	11.82	546.143	99.94% (-0.06%)

The stability of HC Yellow 16 in presence of alkaline peroxide over 45 minutes determined via HPLC, was found to be excellent with 99.99% for 254 nm and 99.84% for 371 nm. No new peaks were found in the HPLC data after 45 minutes for 254 and 371 nm under these conditions. The Applicant concludes that according to the HPLC data, HC Yellow 16 can be considered stable under alkaline peroxide conditions.

Note: A change in the retention time of the HC Yellow 16 is observed for the two effective dye concentration (0.0115% and 0.0255). According to the Applicant, the marginal change in retention time lies within the accepted tolerance of +/-10%. It is mainly caused by the change of the measurement machine to a newer one. The change in retention time of HC Yellow 16 from 12.09 min with the initially submitted new data in submission II to 11.8 min provided with the additional data upon SCCS request, is due to a change in the HPLC system from Dionex Ultimate 3000/ P680 HPLC Pump to Thermo Fischer Vanquish.

<u>Stability of HC Yellow 16 in alkaline peroxide with UVVis</u>
[Batch: Y16_18005_181 206 314]
Alkaline peroxide stability of HC Yellow 16 over 45 min at room temperature (22°C) was determined in a buffered alkaline solution (MEA buffer at pH 10). The sample was analyzed directly after mixing with 6% peroxide solution (t=0), after 15, after 30 and after 45 minutes at room temperature. As reference, the dye solution was mixed with water instead of 6% peroxide solution and analyzed as described above. The results are summarized in Table 12. The maximum of the dye peak for this solvent system was found to be 448 nm, which was used for the evaluation of the absorbance. The stability after 15, 30 and 45 min was determined in reference to the absorbance at 448 nm for t=0 (=100%). The test was repeated 3 times for batch Y16_18005_181 206 314.

Table 12. Alkaline peroxide stability of HC Yellow 16 over 45 min in MEA at pH 10

	Absorbance at 448 nm							
HC Yellow 16 – MEA pH 10	t=0		t=15 min		t=30 min		t=45 min	
Sample 1	1,45510	1,45510	1,45510	1,45510	1,45510	1,45510	1,45510	1,45510
Sample 2	1,48500	1,48500	1,48500	1,48500	1,48500	1,48500	1,48500	1,48500
Sample 3	1,41400	1,41400	1,41400	1,41400	1,41400	1,41400	1,41400	1,41400

According to the Applicant, HC Yellow 16 in alkaline buffered 6% peroxide solution over 45 minutes shows stability greater than 99.25% for both UVVis and HPLC. HC Yellow 16 can be considered stable in alkaline peroxide solution over 45 minutes

General SCCS comments on physicochemical properties-submission II

The SCCS agrees that the purity of the new commercial batches has been improved and that the toxicological data conducted with the old batches are also valid for the new commercial batches.

The method for water solubility determination is unclear because the study report of water solubility determination was not submitted.

3.2 Function and uses

From previous Opinion (SCCS/1568/15)

HC Yellow No. 16 is intended to be used as a direct dye ingredient in oxidative and non-oxidative hair colouring products at on-head concentrations of up to respectively 1% and 1.5%.

3.3 Toxicological evaluation

Additional data provided in submission II

Risk Assessment of the impurities 5B, 7B & 8B in HC Yellow 16

TTC assessment of Impurity 5B and Impurities 7B+8B

As shown in Table 13, the systemic exposure to Impurity 5B and Impurities 7B+8B for both hair dye application scenarios are well below the TTC of 90 µg/person/day and can thus be considered safe.

Table 13. TTC assessment of impurities 5B, 7B & 8B in new commercial batches of HC Yellow 16 under non-oxidative and oxidative conditions using the Cramer Class III threshold of 90 µg/person/day

Product type	SED [µg/person/day]	TTC [µg/person/day]
Impurity 5B		
Non-oxidative hair dye formulation	0.05	90
Oxidative hair dye formulation	0.02	90
Impurities 7B+8B		
Non-oxidative hair dye formulation	0.09	90
Oxidative hair dye formulation	0.04	90

Applicant's conclusion

Three commercial batches of HC Yellow 16 were analysed. The main peak at a retention time of 12.09 min (HPLC analysis) was confirmed to be HC Yellow 16 via comparison of mass and UV-Vis spectra. The quality available in the market could be further purified so that only three impurities, namely 5B, 7B & 8B, remained at low concentrations of 0.124% Impurity 5B and 0.238% Impurity 7B+8B. This high quality is set as standard specification for use.

The maximum allowed on head concentration for HC Yellow 16 is 1.5% under non-oxidative and 1.0% under oxidative conditions. Thus, the consumer is exposed to only 0.00186% or 0.00124% Impurity 5B and to only 0.00357% or 0.00238% Impurity 7B+8B under non-oxidative or oxidative conditions, respectively.

The systemic exposure dose (SED) for a consumer use of a hair colouring product containing 0.124% Impurity 5B and 0.238% Impurity 7B+8B is estimated to be at most 0.05 µg/person/day under non-oxidative conditions and 0.02 µg/person/day under oxidative conditions for Impurity 5B and 0.09 µg/person/day under non-oxidative conditions and 0.04 µg/person/day under oxidative conditions for Impurity 7B+8B. All four calculated SEDs are well below the TTC value of 90 µg/person/day (Cramer Class III). The risk assessment strategy was justified by a state-of-the-art read-across approach using *in silico* predictions to confirm the structural and biological similarity of the Impurities 5B, 7B and 8B and its parent compound HC Yellow 16.

In conclusion, and based on the available data, the quality in the market is safe for use under the present practice of use and concentration and the impurity levels of 5B, 7B and 8B do not pose a health risk to the consumer.

SCCS comment

The SCCS agrees that impurities 5B, 7B and 8B do not pose a health risk to the consumer.

3.3.1 – 3.3.13

Please see SCCS/1568/15 Opinion.

4. CONCLUSION

1. *In light of the data provided, does the SCCS consider HC Yellow 16 safe, when used as an ingredient at 1 % in oxidative hair dye and at 1.5 % in non-oxidative hair dye formulations?*

   In the light of the data provided, SCCS considers that the use of HC Yellow No. 16 (B123) as an ingredient at 1% in oxidative hair dye formulations and at 1.5% in non-oxidative hair dye formulations is safe, considering the specifications of the new commercial batches as described in the submission II file.

2. *Does the SCCS have any further scientific concerns with regard to the use of HC* Yellow *16 in cosmetic products?*

   /

5. MINORITY OPINION

/

6. REFERENCES

See SCCS/1568/15 Opinion

No new reference from Submission II

7. GLOSSARY OF TERMS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158.

8. LIST OF ABBREVIATIONS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158.

APPENDIX I

TTC Assessment of remaining solvents in the new commercial batches

1.5% HC Yellow 16 on head non-ox; 1.0% HC Yellow 16 on head concentration ox

Remaining solvent Methanol:
3000 ppm (=0.3%) or less --> (non-ox) 0.0045% OR (ox) 0.003% methanol on head
Remaining solvent Aceton:
5000 ppm (=0.5%) or less --> (non-ox) 0.0075% OR (ox) 0.005% acetone on head
Remaining solvent Triethylamine:
5000 ppm (=0.5%) or less --> (non-ox) 0.0075% OR (ox) 0.005% triethylamine on head

Worst-case solvent concentrations used for TTC assessment (➔ (non-ox) 0.0075% OR (ox) 0.005% solvent on head)

SED calculation with % -penetration for non-oxidative conditions

Use Concentration in Final Product (C):	0.0075% (1.5% HC Yellow 16 on head concentration contains 0.5% of individual solvent at maximum)
Amount per Application (A):	35 g
Application per day (AD):	1/week = 0.143/day
Retention Factor (R):	0.1
Body Weight (BW):	60 kg
Percutaneous Penetration (P):	50% (used as Default dermal penetration)

SED calculated with %-penetration without intermittent exposure
SED = (C/100 x A x 1000 x 1000 x AD x R x P/100)
18.77 µg/person/day

SED calculation with % -penetration for oxidative conditions

Use Concentration in Final Product (C):	0.005% (1.0% HC Yellow 16 on head concentration contains 0.5% of individual solvent at maximum)
Amount per Application (A):	100 g
Application per day (AD):	1/month = 0.033/day
Retention Factor (R):	0.1
Body Weight (BW):	60 kg
Percutaneous Penetration (P):	50% (used as Default dermal penetration)

SED calculated with %-penetration with intermittent exposure
SED = (C/100 x A x 1000 x AD x 1000 x R x P/100)
8.25 µg/person/day

Methanol, acetone and triethylamine are not classified as CMR and Cramer Classification resulted in Cramer Class I (low) for both solvents with Chemtunes.ToxGPS. As an overarching worst-case assessment for all contained solvents the Applicant used Cramer Class III (90 µg/person/day) and the highest specified solvent concentration for their calculations.

APPENDIX II

Risk Assessment of the impurities 5B, 7B & 8B in HC Yellow 16

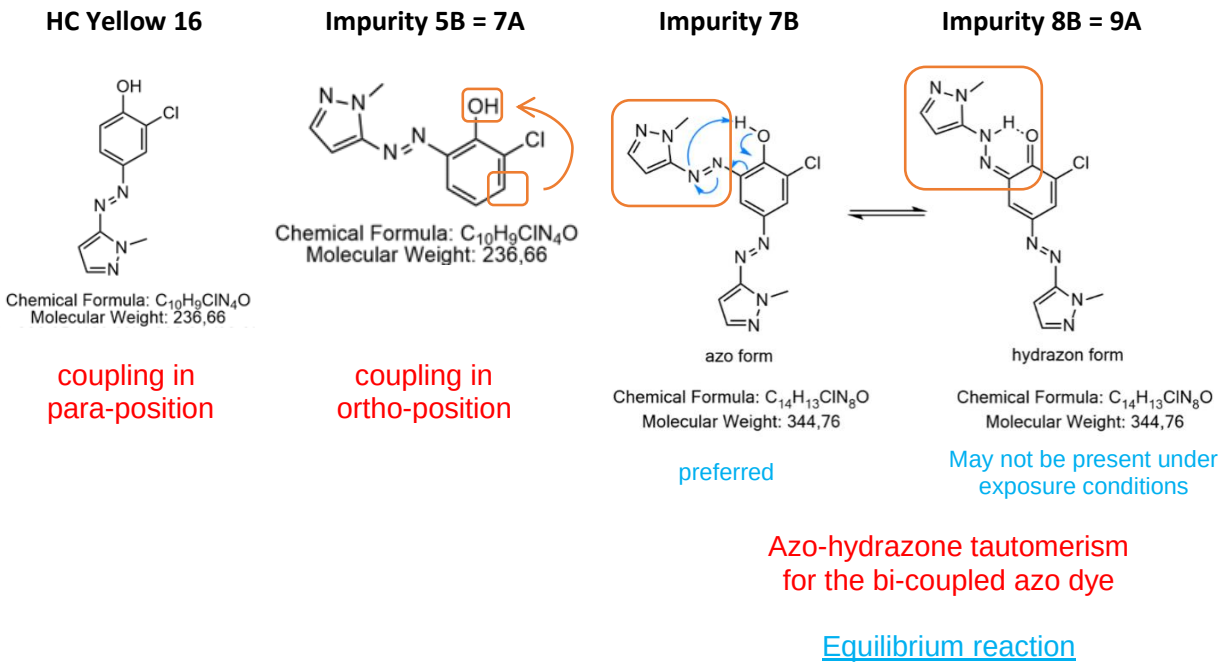
New market specification: Relevant impurities 5B, 7B & 8B are only present at the following levels:

	Conc. in new commercial batches	
Impurity identification	HPLC at 254 nm	HPLC at 371 nm
Impurity 5B (= 7A)	0.056%	**0.124%**
Impurity 7B	**0.026%**	Not found
Impurity 8B (= 9A)	0.172%	**0.212%**

Bold marked concentrations used as worst-case for the risk assessment.

Figure 7.

Proposed structure of impurities 5B, 7B & 8B as explained above and comparison to HC Yellow 16:



Since no toxicological data could be found for impurities 5B, 7B & 8B and only a low amount of these impurities was detected in the dye, the use of the TTC approach is indicated. By using NAMs, like state-of-the-art *in silico* predictions, and read-across the applicant aimed to identify which TTC threshold is appropriate to use for the risk assessment.
All structures were classified as Cramer Class III.
A TTC value of 90 µg/day can be considered as appropriate if the structures have no genotoxic potential. To provide more evidence that Cramer Class III is sufficient for a safety assessment of impurities 5B, 7B & 8B and that no genotoxic potential must be assumed for the structures, the Applicant performed a state-of-the-art read-across to the data-rich parent compound HC Yellow 16.
To justify the read-across approach and to decide whether the Applicant can use the toxicological profile of HC Yellow 16 as a basis for the safety assessment of impurities 5B,

7B & 8B, they compared the structural and biological similarity of HC Yellow 16 to the impurities 5B, 7B & 8B.

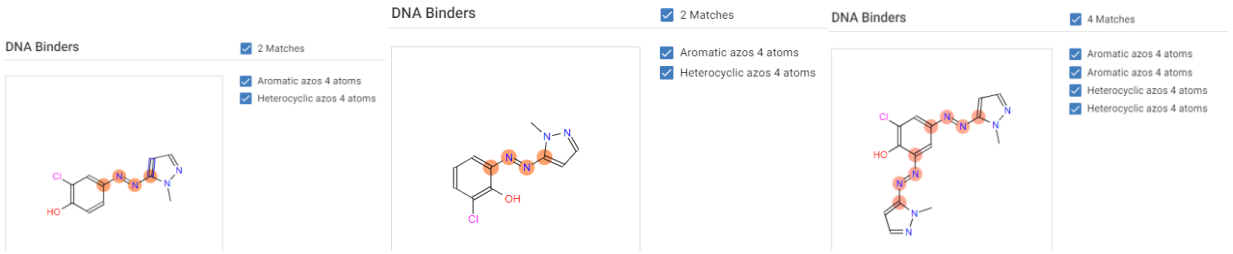
In a first step, they calculated the pairwise similarity using the Tanimoto coefficient, and the chemical fingerprints incorporated in the *in silico* tools *AMBIT,* developed by Cefic-LRI (https://ambitlri.ideaconsult.net/tool2), and *Chemtunes.ToxGPS*. The structural similarity was predicted to be very high with values between 86 - 93%.

In a second step, they assessed the biological similarity of all structures using chemotype predictions for DNA binders and toxicological endpoint predictions for Bacterial Reverse Mutagenicity, *In vitro* Chromosome Aberration and *In vivo* Micronucleus made by *Chemtunes.ToxGPS*.

All predicted genotoxicity endpoints made with *Chemtunes.ToxGPS* are negative for the impurities and HC Yellow 16, indicating a high biological similarity in addition to the high structural similarity.

In addition, the two chemotype matches found for DNA Binders for HC Yellow 16 trace back to two chemical structures, namely the aromatic azos (4 atoms) and heterocyclic azos (4 atoms), which are equally contained in both, the impurities 5B & 7B and HC Yellow 16 (see Figure 8). Impurity 7B contains the same alerts twice (four instead of two) because of the bi-azo coupled nature of the reaction product but no different/further chemotype alerts.

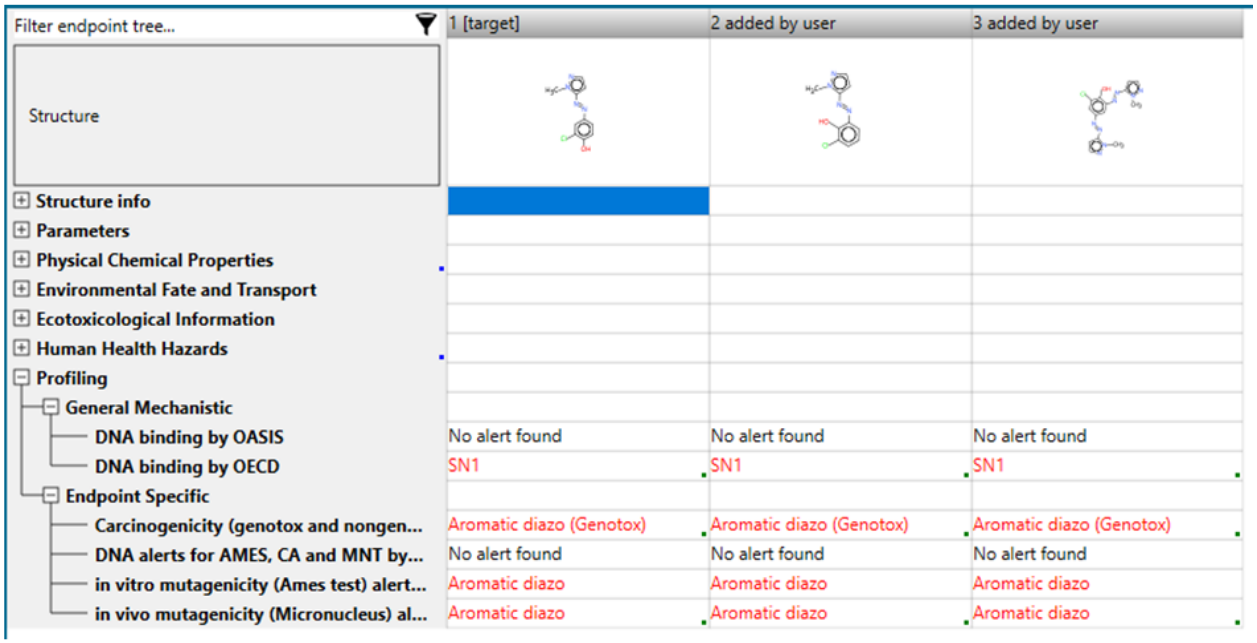
Figure 8. Chemotype matches for HC Yellow 16 and impurities 5B & 7B using Chemtunes.ToxGPS (Screenshot)



HC Yellow 16 | Impurity 5B | Impurity 7B

Moreover, the Applicant searched for mechanistic or structural alerts regarding genotoxicity using general mechanistic (DNA binding by OASIS; DNA binding by OECD) and endpoint sprecific (Carcinogenicity (genotox and nongenotox) alerts by ISS; DNA alerts for AMES, CA and MNT by OASIS; *in vitro* mutagenicity (Ames test) alerts by ISS; *in vivo* mutagenicity (Micronucleus) alerts by ISS; Protein binding alerts for Chromosomal aberration by OASIS) profilers via OECD QSAR Toolbox to further assess the biological similarity of HC Yellow 16 and the impurities 5B & 7B.

Figure 9: Mechanistic or structural alerts regarding genotoxicity for HC Yellow 16 and impurities 5B & 7B using OECD QSAR Toolbox (Screenshot)



Filter endpoint tree...	1 [target]	2 added by user	3 added by user
Structure			
Structure info			
Parameters			
Physical Chemical Properties			
Environmental Fate and Transport			
Ecotoxicological Information			
Human Health Hazards			
Profiling			
General Mechanistic			
DNA binding by OASIS	No alert found	No alert found	No alert found
DNA binding by OECD	SN1	SN1	SN1
Endpoint Specific			
Carcinogenicity (genotox and nongen...	Aromatic diazo (Genotox)	Aromatic diazo (Genotox)	Aromatic diazo (Genotox)
DNA alerts for AMES, CA and MNT by...	No alert found	No alert found	No alert found
in vitro mutagenicity (Ames test) alert...	Aromatic diazo	Aromatic diazo	Aromatic diazo
in vivo mutagenicity (Micronucleus) al...	Aromatic diazo	Aromatic diazo	Aromatic diazo

As shown in Figure 9 the same mechanistic and structural alerts regarding genotoxicity were found for HC Yellow 16 and impurities 5B & 7B with OECD QSAR TB giving further evidence for a high biological similarity in addition to the high structural similarity. In addition, the found endpoint specific alerts fit with the genotoxic endpoint predictions of *Chemtunes.ToxGPS*.

Based on the high structural and biological similarity, a read-across to the toxicological and dermal penetration data of the parent compound HC Yellow 16 can be justified for impurities 5B, 7B & 8B, according to the applicant.

As described in Submission I (2014) and accepted by the SCCS in its opinion SCCS/1568/15, no genotoxic potential must be assumed for HC Yellow 16. HC Yellow 16 (T44P2) was investigated sufficiently in four *in vitro* genotoxicity tests and one *in vivo* genotoxicity test covering all three endpoints of genotoxicity (gene mutations, structural (clastogenicity) and numerical (aneugenicity) chromosome aberrations).

In vitro, HC Yellow 16 did not induce gene mutations in five bacteria strains and in one mammalian cell system and did also not induce micronuclei in Human lymphocytes in the absence or presence of metabolic activation, respectively. *In vivo*, the Bone marrow micronucleus test in the rat with HC Yellow 16 led to no biologically relevant increase in the number of bone marrow cells with micronuclei at any dose tested. Thus, HC Yellow 16 was also shown to be non-mutagenic *in vivo*.

The experimental results confirm the inconspicuous toxicological endpoint predictions for Bacterial Reverse Mutagenicity, *In vitro* Chromosome Aberration and *In vivo* Micronucleus made by the *in silico* tools *Chemtunes.ToxGPS* & *OECD QSAR TB* and overrules the chemotype alerts for the aromatic azos (4 atoms), heterocyclic azos (4 atoms) and SN1 reaction for DNA binding in all structures (= HC Yellow 16 and impurities 5B, 7B & 8B).

In summary, according to the Applicant the high structural and biological similarity of HC Yellow 16 and the impurities 5B, 7B & 8B justifies the read-across approach and the assumption that it is very unlikely that impurities 5B, 7B & 8B have a genotoxic potential.

Therefore, the Applicant decided to use the Cramer Class III value of 90 µg/person/day for the risk assessment of impurities 5B, 7B & 8B using the TTC approach. Calculation is based on the method published by Kroes *et al.* ("Application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients", 2007, Food and Chemical Toxicology, (45), 2533-2562) and the SCCP/1171/08.

SED calculation of Impurity 5B in new commercial batches of HC Yellow 16

SED calculation with % -penetration for non-oxidative conditions

Use Concentration in Final Product (C):	0.00186% (1.5% HC Yellow 16 on head concentration contains 0.124% Impurity 5B at maximum)
Amount per Application (A):	35g
Application per day (AD):	1/week = 0.143/day
Retention Factor (R):	0.1
Body Weight (BW):	60 kg
Percutaneous Penetration (P):	0.5% (used as worst-case due to structural similarity to HC Yellow 16 (dermal penetration under non-oxidative conditions: 0.281% (MW + SD) [SCCS/1568/15])

SED calculated with %-penetration without intermittent exposure

SED = (C/100 x A x 1000 x 1000 x AD x R x P/100)

0.05 µg/person/day

SED calculation with % -penetration for oxidative conditions

Use Concentration in Final Product (C):	0.00124% (1.0% HC Yellow 16 on head concentration contains 0.124% Impurity 5B at maximum after mixing with H2O2 (1/1))
Amount per Application (A):	100 g
Application per day (AD):	1/month = 0.033/day
Retention Factor (R):	0.1
Body Weight (BW):	60 kg
Percutaneous Penetration (P):	0.5% (used as worst-case due to structural similarity to HC Yellow 16 (dermal penetration under oxidative conditions: 0.250% (MW + SD) [SCCS/1568/15])

SED calculated with %-penetration with intermittent exposure

SED = (C/100 x A x 1000 x AD x 1000 x R x P/100)

0.02 µg/person/day

SED calculation of Impurities 7B+8B in new commercial batches of HC Yellow 16

Since the chemical structures of impurity 7B and 8B are nearly identical (while also being under an equilibrium reaction) and we assume a nearly identical toxicological activity for them, we added the impurity concentrations up in order to assess their combined risk potential: 0.212% Impurity 8B + 0.026% Impurity 7B = 0.238% Impurity 7B+8B.

SED calculation with % -penetration for non-oxidative conditions

Use Concentration in Final Product (C):	0.00357% (1.5% HC Yellow 16 on head concentration contains 0.238% Impurities 7B+8B at maximum)
Amount per Application (A):	35g
Application per day (AD):	1/week = 0.143/day
Retention Factor (R):	0.1
Body Weight (BW):	60 kg
Percutaneous Penetration (P):	0.5% (used as worst-case due to structural similarity to HC Yellow 16 (dermal penetration

	under non-oxidative conditions: 0.281% (MW + SD) [SCCS/1568/15])

SED calculated with %-penetration without intermittent exposure

SED = (C/100 x A x 1000 x 1000 x AD x R x P/100)

0.09 µg/person/day

<u>SED calculation with % -penetration for oxidative conditions</u>

Use Concentration in Final Product (C)	0.00238% (1.0% HC Yellow 16 on head concentration contains 0.238% Impurities 7B+8B at maximum after mixing with H_2O_2 (1/1))
Amount per Application (A):	100 g
Application per day (AD):	1/month = 0.033/day
Retention Factor (R):	0.1
	Body Weight (BW): 60 kg
Percutaneous Penetration (P):	0.5% (used as worst-case due to structural similarity to HC Yellow 16 (dermal penetration under oxidative conditions: 0.250% (MW + SD) [SCCS/1568/15])

SED calculated with %-penetration with intermittent exposure

SED = (C/100 x A x 1000 x AD x 1000 x R x P/100)

0.04 µg/person/day
