Scientific Committee on Consumer Safety

SCCS

SCIENTIFIC ADVICE on HC Blue 18 (Colipa No. B122)

Submission II

The SCCS adopted this document by written procedure

on 27 April 2023

ACKNOWLEDGMENTS

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1. ABSTRACT

The SCCS concludes the following:

1. In light of the data provided, does the SCCS consider HC Blue 18, safe when used in non-oxidative as well as in oxidative hair dye formulations up to a maximum on-head concentration of 0.35 %?

In light of the new physicochemical data provided, SCCS considers that the use of HC Blue 18 as an ingredient in non-oxidative as well as in oxidative hair dye formulations up to a maximum on-head concentration of 0.35% is safe.

2. Does the SCCS have any further scientific concerns with regard to the use of HC Blue 18 in cosmetic products?

SCCS considers HC Blue 18 as a moderate sensitiser.

Keywords: SCCS, scientific advice, hair dye, HC Blue 18, Colipa No B122, Submission II, CAS No. 1166834-57-6

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2. MANDATE FROM THE EUROPEAN COMMISSION

Background

The ingredient with the INCI name 'HC Blue 18' and chemical name '3-[(E)-(3-chloro-4-hydroxyphenyl)diazenyl]-2,1-benzisothiazole-5-sulfonamide' (CAS No. 1166834-57-6/852356-91-3, EC No. -/-) is used as an ingredient in oxidative and non-oxidative hair dye formulations.

In 2014, the Commission services received a dossier from industry to support the safe use of 'HC Blue 18' in cosmetic products. In its corresponding opinion *(SCCS/1560/15)*, the SCCS concluded that *'the use of HC Blue 18 (B122) as an ingredient in non-oxidative as well as in oxidative hair dye formulations at a maximum concentration of 0.35% on the head is safe'.* In addition, the SCCS stated that '*The purity of HC Blue 18 and impurities in it are not adequately quantified. Details on impurities will be submitted at a later date by the applicant'*.

With the current submission (i.e., submission II), received in October 2022, the applicant requests to assess the safety of 'HC Blue 18' in view of the newly provided information on its purity, when used as an ingredient in non-oxidative as well as in oxidative hair dye formulations at a maximum concentration of 0.35% on the head.

Terms of reference

1. In light of the data provided, does the SCCS consider HC Blue 18, safe when used in non-oxidative as well as in oxidative hair dye formulations up to a maximum on-head concentration of 0.35 %?

2. Does the SCCS have any further scientific concerns with regard to the use of HC Blue 18 in cosmetic products?

3. SCIENTIFIC ADVICE

3.1 Physicochemical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

HC Blue 18

3.1.1.2 Chemical names

3-[(E)-(3-chloro-4-hydroxyphenyl)diazenyl]-2,l-benzisothiazole-5- sulfonamide

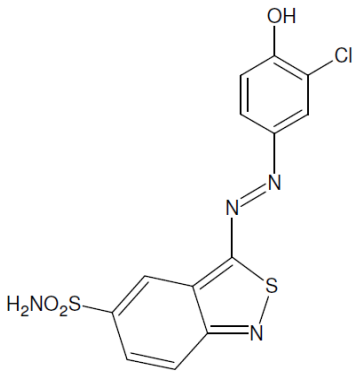
3.1.1.3 Trade names and abbreviations

FPK-145

3.1.1.4 CAS / EC number

CAS: 1166834-57-6
EC: /

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

$C_{13}H_9ClN_4O_3S_2$

3.1.2 Physical form

Red powder

3.1.3 Molecular weight

Molecular weight: 368.82 g/mol

3.1.4 Purity, composition, and substance codes

New data

According to the Applicant, current commercial batches are of even better quality than the batches used in the previous submission, while existing toxicological data is still valid. The manufacturer was able to improve the already high purity of HPLC Blue 18 and this is reflected in a further reduction in concentration and/or number of impurities in the current commercial batches of HC Blue 18 compared to the batches Y6-9, 04040601, T9611-9612 and 11-001 used in the previous submission.

As these old batches no longer represent the current commercial quality of HC Blue 18 and only the current commercial quality will be available on the market, the Applicant decided to use three current commercial batches (Table 1) when submitting the requested data.

Table 1. Overview of the 4 batches used in previous submission ("Old Batches") and the 3 batches used for providing the requested data ("New Commercial Batches").

Old Batches	New Commercial Batches
Y6-9	B18_18003_190 881 100
04040601	B18_18004_190 881 097
T9611-9612	B18_18005_190 220 120
11-001 -	

Chemical characterisation of HC Blue 18 was performed in three new commercial batches (Table 1) by H^1-NMR, ^{13}C-NMR and FT-IR.

All ^1H 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.

All FT-IR spectra of HC Blue 18 showed the same absorption bands for each compound unique fingerprint area between 1600 and 400 nm^{-1}.

Purity of HC Blue-18 in old and new commercial batches is presented in Table 2.

SCCS comment

The empirical formula of HC Blue 18 is $C_{13}H_9ClN_4O_3S_2$, and this is reflected by the interpretation of the received ^1H and ^{13}C NMR spectra.

3.1.5 Impurities / accompanying contaminants

New data

HPLC-PDA analysis of impurities

The HPLC method for the impurity analysis of HC Blue 18 was further improved by increasing the buffer capacity from 0.1% to 0.3%. With the current method, the Applicant achieved consistent retention times over all conducted tests. All HPLC measurements were conducted on the same HPLC instrument to avoid retention times shift due differences between HPLC instruments. Peak purity was determined by comparing the found UV-Vis spectrum at different retention times within the peak of the HC Blue 18 peak as depicted in Figure 1. All spectra show the same characteristic double absorption maximum at 452 and 575 nm. No additional peaks are visible

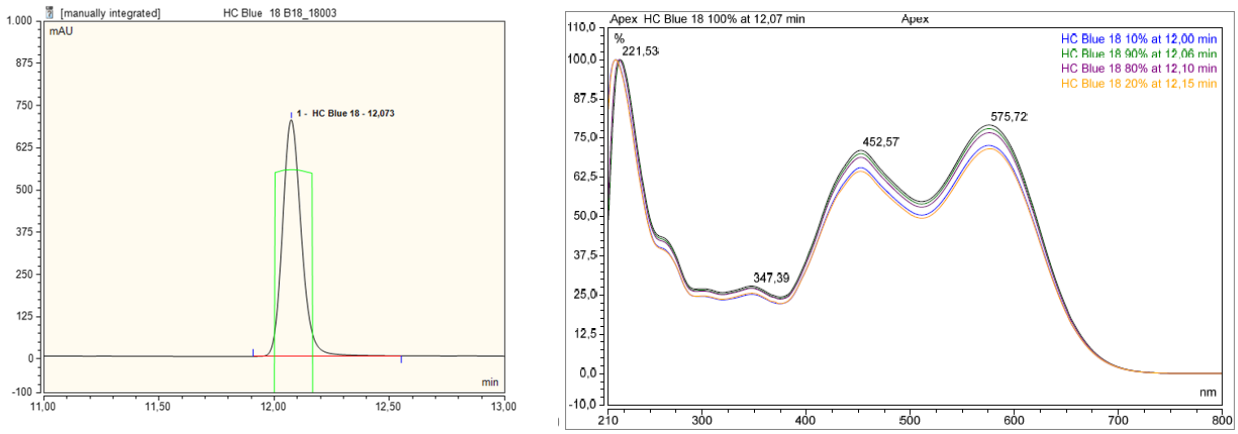


Figure 1. Determination of peak purity of HC Blue 18 determined by the UV spectra detected at different points in the HPLC peak

The LOD for HC Blue 18 with the aforementioned HPLC method was determined to be below 21.6 ppb (=0.00000216 %).
Purity and impurities of HC Blue 18 were determined for all old batches and new commercial batches, using the same method and HPLC instrument at both 254 nm and 575 nm (absorption maximum of anionic HC Blue 18).

Table 2. Summary of the relative % area obtained from the HPLC data of HC Blue 18 at 254 nm

At 254 nm	**Old batches**				**New commercial batches**		
	T9611_9612	**11_001**	**04040601**	**Y6-9**	**B18_18003**	**B18_18004**	**B18_18005**
Impurity 1A 11.03-11.04	0.160%	0.017%		0.208%			
Impurity 2A 11.43			0.017%				
Impurity 3A 11.53-11.54	0.037%	0.129%					
HC Blue 18 12.25-12.28	99.576%	99.787%	99.917%	99.578%	100.000%	100.000%	100.000%
Impurity 4A 13.16-13.19	0.119%	0.067%					
Impurity 5A 14.02				0.123%			
Impurity 6A 14.36-14.39	0.108%		0.050%	0.040%			
Impurity 7A 16.85			0.017%	0.051%			
Total	100%	100%	100%	100%	100%	100%	100%

According to the Applicant, purity for the old batches was found to be already high with 99.58+% within 4 batches at 254 nm. For the new commercial batches, purity had been even further improved. While in the old batches, small amounts of seven different impurities could be found at 254 nm, no impurities could be detected in the new commercial batches using the same method.

Table 3. Summary of the relative % area obtained from the HPLC data of HC Blue 18 at 575 nm

At 575 nm	**Old batches**				**New commercial batches**		
	T9611_9612	**11_001**	**04040601**	**Y6-9**	**B18_18003**	**B18_18004**	**B18_18005**
Impurity 1B 10.52-11.54	0.017%	0.008%	0.009%	0.006%			
Impurity 2B 11.03-11.05	0.087%	0.028%	0.028%	0.164%			
Impurity 3B 11.32-11.35	0.142%	0.067%	0.060%	0.008%	0.016%	0.016%	0.019%
Impurity 4B 11.54-11.55	0.007%	0.011%	0.009%				
Impurity 5B 11.80-11.82		0.004%					
HC Blue 18 12.25 – 12.28	99.660%	99.815%	99.845%	99.681%	99.984%	99.984%	99.981%
Impurity 6B 13.17-13.20	0.029%	0.023%					
Impurity 7B 14.02			0.009%	0.125%			
Impurity 8B 14.35-14.40	0.036%		0.019%	0.014%			
Impurity 9B 16.78-16.84	0.022%	0.043%	0.021%				
Total	100%	100%	100%	100%	100%	100%	100%

According to the Applicant, the results of HPLC analysis at 575 nm show that it was possible to improve the already high purity of the old batches of 99.66+% to 99.98+% in the new commercial batches. Additionally, the number of impurities could be reduced from seven to one in the new commercial batches.
The found impurity in the new commercial batches at ~11.3 min (Impurity 3B) was also present in the old batches. The highest amount found in the old batches for Impurity 3B was 0.142% while the highest concentration found in the new commercial batches is 0.019%. As the Impurity 3B found in the old commercial batches at 575 nm is also present in the new batches, but with a noticeable lower concentration, the Applicant concluded that the toxicological data conducted with the old batches is also valid for the new commercial batches.

Ash, water, and heavy metal content of HC Blue 18
Data for water and heavy metal content, for the remaining solvent, and for residue on ignition were taken from the specification sheet delivered for each batch by the dye manufacturer. Heavy metal content, remaining solvent, and residue on ignition were determined with standard methods by the manufacturer.

Table 4. Impurity data for each new HC Blue 18 batch as per manufacturers specification

Impurity	Specification	B18_18003 190 881 100	B18_18004 190 881 097	B18_18005 190 220 120
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
Remaining solvent Methanol	3000 ppm or less	157 ppm	176 ppm	235 ppm
Remaining solvent	720 ppm or less	117 ppm	75 ppm	122 ppm

THF				
Water content	1.0% or less	0.06%	0.02%	0.05%
Residue on ignition	1.0% or less	0.08%	0.02%	0.01%

The water content was determined using the Karl-Fischer method. Water and heavy metal content, the remaining solvent, and residue on ignition are well below the specified maximum amount in all three batches (Table 4).

Impurity Characterization for Commercial Batches

Impurity analysis of new commercial batches was performed with HPLC and LC-MS measurements. Only impurities found in both old batches and the new commercial batches are presented.

HPLC-PDA analysis

At 575 nm, Impurity 3B could be found in the HPLC analysis of the new commercial batches at ~11.3 minutes (Table 3). As shown in Table 3, this impurity is also present in the old batches. Impurity 3B is present in all batches when using 575 nm as the detection wavelength, and shows an absorption spectrum similar to the anionic form of HC Blue 18 with a single absorption maximum between 573-583 nm. According to the Applicant, the very low concentration of the impurity results in a weak absorption that makes peak analysis difficult

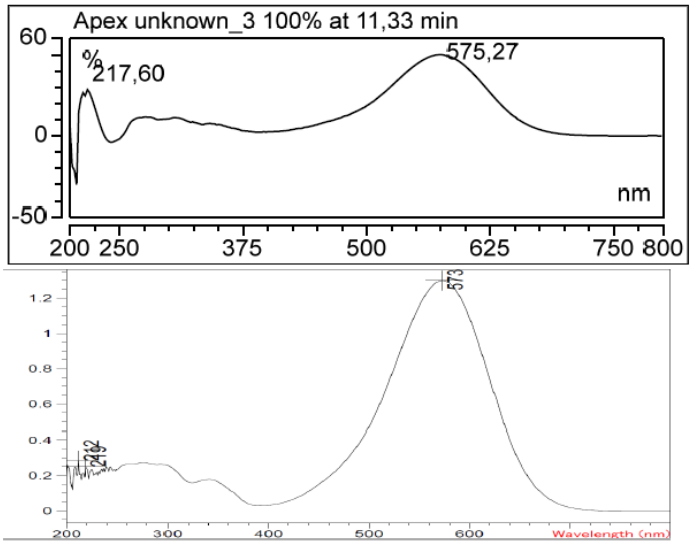


Figure 2. Comparison of the UV-Vis spectrum of Impurity 3B (above) and the anionic form of HC Blue 18 (below)

According to the Applicant, the spectrum of Impurity 3B shows that a chromophore system must be present within the molecule that is very similar to the chromophore system of HC Blue 18, considering the possible pathways that such a chromophore system can form under the given chemical reaction. In Figure 3, the azo-coupling reaction of HC Blue 18 and possible side reactions are shown:

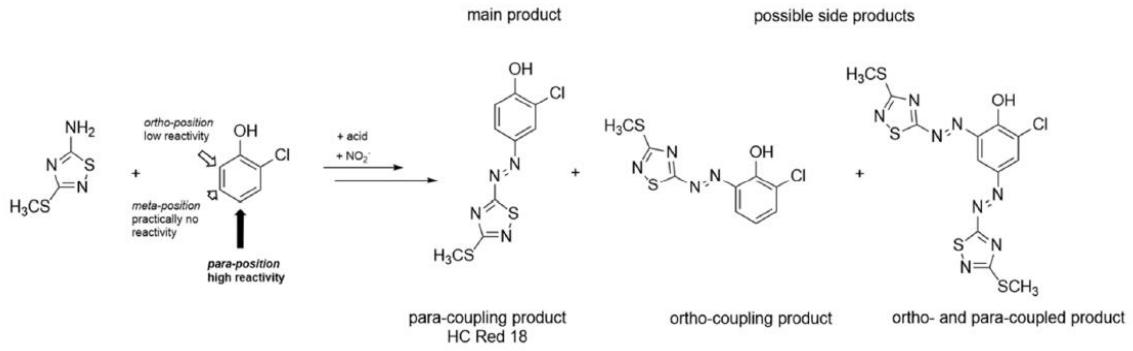


Figure 3. Schematic depiction of azo-coupling reaction including known side products.

According to the Applicant, the main reaction product of this azo-coupling reaction is the para-coupling product (= HC Blue 18). Also possible, but due to sterical hindrance far less likely, is the azo-coupling reaction in the ortho-position of orthochlorophenol. The ortho-coupled azo-dye does have the same molar mass as HC Blue 18, but the chromophore system is changed, as it is shorter and therefore results in a hypsochromic shift (= shorter wavelength) of the absorption maximum. As Impurity 3B has a very similar absorption maximum as HC Blue 18, the Applicant concludes that it cannot be the ortho-coupled product. 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 Blue 18. This dye has two chromophore systems (ortho-azo dye and para-azo-dye) in one molecule, leading to two different absorption maxima in the spectrum; one for each chromophore. As this was not observed in the spectrum of Impurity 3B, the Applicant concluded that this cannot be the structure of this impurity.
According to the Applicants' conclusions, analysis of the HPLC data of HC Blue 18 shows that a single impurity, Impurity 3B, is present. To achieve the found absorption wavelength of Impurity 3B, and taking into account the chemical reaction that formed this impurity, the Applicant concluded that the impurity has a very similar chromophore system, based on a para coupled azo-dye molecular structure, as HC Blue 18.

LC-MS analysis of commercial Batches
Representative batch B18_18003_190 881 100 was analysed with LC-MS, using a method that was adapted from the HPLC-analysis and further optimised for LC-MS measurement. Due to the different hardware for LC-MS and HPLC analysis, the adoption of the HPLC method to the LC system, and a necessary change of column dimensions, an unavoidable change in retention time is observed. Thus, retention times between HPLC and LC-MS will not be compared for this analysis.
The main peak of HC Blue 18 was found at a retention time of 6.89 min, while Impurity 3B has a retention time of 5.17 min.

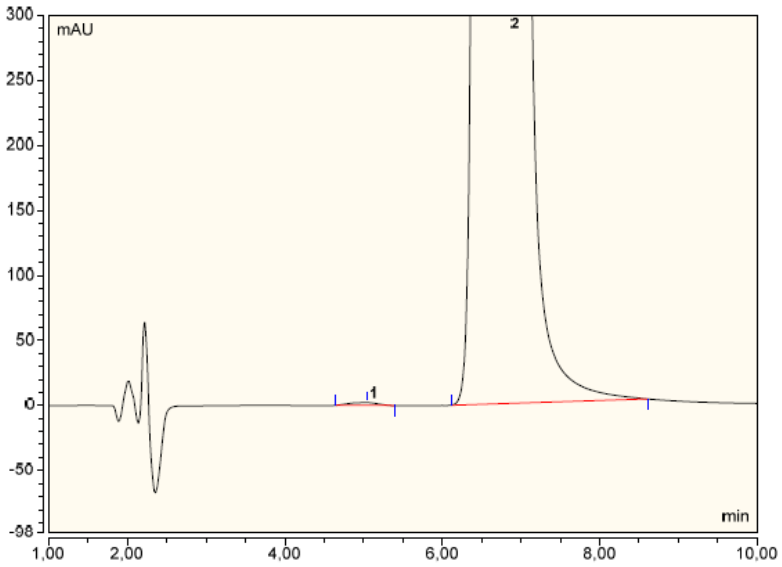


Figure 4. LC-UV chromatogram of HC Blue 18 [2] with Impurity 3B [1]

The total ion current (TIC) chromatogram (Figure xxx) shows the same two peaks as the obtained LC-UV chromatogram.

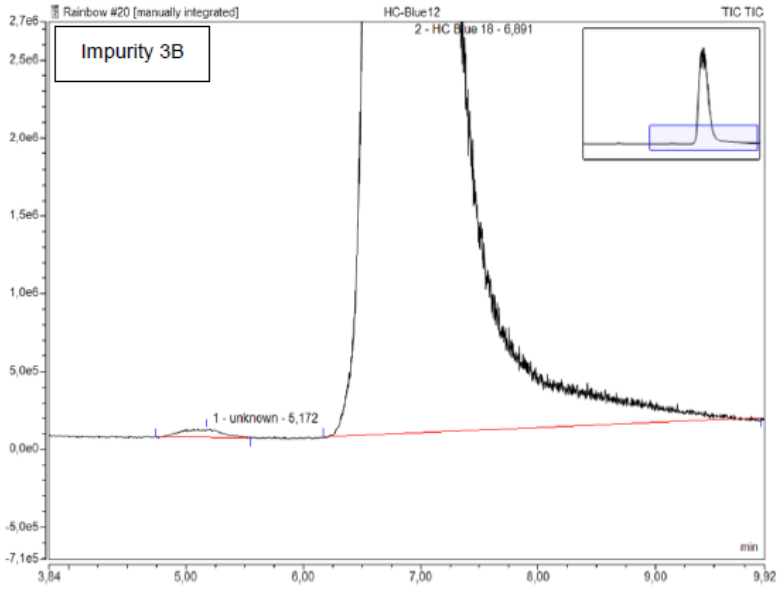


Figure 5. TIC-MS spectra of HC Blue 18 [2] with Impurity 3B [1]

For the MS analysis of Impurity 3B, a high sample weight was necessary to achieve the necessary concentration due to the very low concentration of Impurity 3B in HC Blue 18 (<0.02%). However, this inevitably leads to the jagged HC Blue 18 peak as seen above, due to the resulting high concentration. This has no negative influence on the actual mass detection of the peak.

Analysis of HC Blue 18 peak at 6.89 min:
The obtained data for MS as well as UV-Vis matches the expected values for HC Blue 18, having a molar mass of M=368.81 g/mol and two absorption maxima of Absmax = 453 and 575 nm. The expected base peak in ESI negative mode of [M-1]- = 367 could be confirmed.

Additionally, the calculated characteristic isotope pattern matches the one measured, further providing evidence that the peak is indeed HC Blue 18.

Analysis of Impurity 3B peak at 5.17 min:
The base peak for Impurity 3B, was found to be [M-1]- = 401. Compared to the base peak of HC Blue 18 of [M-1]- = 367, this is an increase in mass of +34. This MS spectrum fits to a structure of HC Blue 18 where a hydrogen is substituted by a chlorine. Also, the characteristic isotope pattern of HC Blue 18+Cl matches the calculated values for such a structure.

According to the Applicant, an explanation for the found UV-Vis and mass spectra would be the chemical structure as shown in Figure 6.

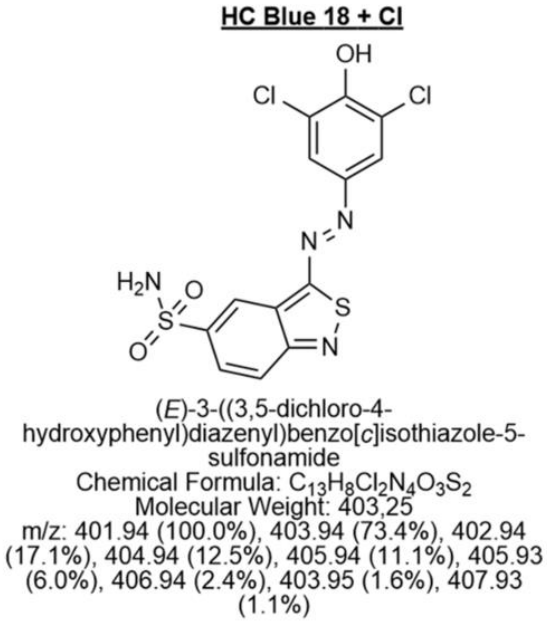


Figure 6. Suggested structure of Impurity 3B based on the found data in UV-Vis and mass analysis

In summary, analysis of the UV-Vis spectra obtained during the HPLC measurement indicates a very similar structure of the Impurity 3B as the para-coupled azo dye HC Blue 18. In the LC-MS, the main peak with a retention time of 6.89 min was confirmed to be HC Blue 18 through a comparison of mass analysis and UV-Vis spectra. The mass of impurity 3B was found to match the structure of (E)-3-((3,5-dichloro-4-hydroxyphenyl)diazenyl)-benzo[c]isothiazole-5-sulfonamide. This would also match the obtained UV-Vis spectrum and the observed bathochromic shift of the absorption maximum.

SCCS comment
The SCCS agrees that the purity of the new commercial batches has been improved and only one impurity is detected at 575 nm (Impurity 3B).
The SCCS agrees that Impurity 3 B was chemically characterised by the Applicant and matches the structure of (E)-3-((3,5-dichloro-4-hydroxyphenyl)diazenyl)-benzo[c]isothiazole-5-sulfonamide.

TTC approach on Impurity 3B

The Applicant predicted the Cramer Class for both structures, HC Blue 18 and Impurity 3B, using the *in silico* tools Chemtunes.ToxGPS developed by MN-AM and OECD QSAR Toolbox

(Version 4.4.1; https://qsartoolbox.org/) developed by LMC. Both structures were classified as Cramer Class III via both tools.
Thus, according to the Applicant, a TTC value of 90 µg/day can be considered as appropriate if the structure has no genotoxic potential. To further demonstrate that Cramer Class III is sufficient for a safety assessment of Impurity 3B and that no genotoxic potential must be assumed for the structure, the Applicant performed a state-of-the-art read-across to the data-rich parent compound HC Blue 18.
To justify the read-across approach and to decide whether the toxicological profile of HC Blue 18 could be used as a basis for the safety assessment of Impurity 3B, a comparison of the structural and biological similarity of HC Blue 18 to Impurity 3B was performed by the Applicant.
In a first step, the Applicant 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 70 - 100%.
In a second step, the biological similarity of both structures was assessed, using chemotype predictions made by Chemtunes.ToxGPS for DNA binders and toxicological endpoint predictions for Bacterial Reverse Mutagenicity, *in vitro* Chromosome Aberration and *in vivo* Micronuclues. Even if the toxicological endpoint prediction for Bacterial Reverse Mutagenicity could not be calculated for Impurity 3B, all other predictions made by Chemtunes.ToxGPS are the same for Impurity 3B and HC Blue 18, indicating a high biological similarity in addition to a high structural similarity. Furthermore, the toxicological endpoint predictions for *in vitro* Chromosome Aberrration and *in vivo* Micronuclues revealed no alert for a genotoxic potential for any of the two structures. In addition, the two chemotype matches found for DNA Binders for both structures trace back to the same chemical structure, namely the azo-bond (N=N double bond), which is equally contained in both Impurity 3B and HC Blue 18.
Moreover, the Applicant has searched for mechanistic or structural alerts regarding genotoxicity using general mechanistic alerts (DNA binding by OASIS; DNA binding by OECD) and endpoint specific (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 Blue 18 and Impurity 3B. The same mechanistic and structural alerts regarding genotoxicity were found for HC Blue 18 and Impurity 3B, indicating also a high biological similarity in addition to a high structural similarity.
According to the Applicant, based on the high structural and biological similarity, a read-across to the toxicological data of the parent compound HC Blue 18 can be justified for Impurity 3B. As described in Submission I (2005) and accepted by the SCCS in its Opinion SCCS/1560/15, no genotoxic potential must be assumed for HC Blue 18. Thus, the high structural and biological similarity of both compounds justifies the proposed read-across approach and the assumption that it is very unlikely that Impurity 3B has a genotoxic potential.

Therefore, the Applicant has used the Cramer Class III value of 90 µg/person/day for the risk assessment of Impurity 3B, using the TTC approach.
The 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 with % -penetration for non-oxidative conditions

Use Concentration in Final Product (C):	0.00007% (0.35% HC Blue 18 on head concentration contains 0.02% Impurity 3B 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):	10% (used as worst-case due to structural similarity to HC Blue 18 (dermal penetration under non-oxidative conditions: 1.57% [SCCS/1560/15])
SED calculated with %-penetration without intermittent exposure. SED = (C/100 x A x 1000 x 1000 x AD x R x P/100) **0.04 µg/person/day**	

SED calculation with % -penetration for oxidative conditions

Use Concentration in Final Product (C):	0.00007% (0.35% HC Blue 18 on head concentration contains 0.02% Impurity 3B at maximum after mixing with H_2O_2 (1/1))
Amount per Application (A):	100g
Application per day (AD):	1/month = 0.033/day
Retention Factor (R):	0.1
Body Weight (BW):	60 kg
Percutaneous Penetration (P):	10% (used as worst case due to structural similarity to HC Blue 18 (dermal penetration under oxidative conditions: 0.225% [SCCS/1560/15])
SED calculated with %-penetration without intermittent exposure. SED = (C/100 x A x 1000 x AD x 1000 x R x P/100) **0.02 µg/person/day**	

Thus, the Applicant used the SEDs of 0.04 µg/person/day for non-oxidative conditions and 0.02 µg/person/day for oxidative conditions for the risk assessment and compared them to the TTC value of 90 µg/person/day.

Table 5. TTC assessment of Impurity 3B in HC Blue 18 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]**	**Margin of Exposure**
Considering the fact that the dye is rinsed off after 30 minutes (RF = 0.01)			
Non-oxidative hair dye formulation	0.04	90	2,250
Oxidative hair dye formulation	0.02	90	4,500

According to the Applicant, as shown in Table 5, the systemic exposure to Impurity 3B for both hair dye application scenarios is well below the TTC of 90 µg/person/day.

SCCS comments on TTC approach
The SCCS agrees that both HC Blue 18 and the impurity 3B belong to Cramer Class III; the impurity is analogous with HC Blue 18 as indicated by the high Tanimoto coefficient. Read-across results – and also QSAR results – indicate that the close analogues are negative for genotoxicity. Hence, it can be considered that impurity 3B is not of genotoxicological concern. Based on the TTC assessment of Impurity 3B, the calculated SED values are below the (external) TTC.

Taken from SCCS/1560/15

Batches used in respective toxicological studies

Lot 04040601	T-9611-9612	11-001
- Eye irritation - Skin irritation - Micronucleus Assay in bone marrow of the mouse - Local Lymph Node Assay (LLNA)	- Ames Assay - Micronucleus assay in human lymphocytes - Gene mutation assay in Chinese hamster V79 cells in vitro - In vitro dermal delivery of cream 0.7% FPK-145 under oxidative conditions - 14 day oral repeated dose toxicity study	- Teratogenicity - Subchronic toxicity - 90 day oral toxicity study - In vitro dermal delivery of cream 0.35% FPK-145 under non-oxidative conditions

SCCS comment
The SCCS agrees that the current commercial batches of the test substance are of better quality than the batches used in the previous submission, while the existing toxicological data are still valid.

3.1.6 Solubility

Taken from SCCS/1560/15

Water: 1% (pH9)
DMSO: > 10%
Ethanol < 1%

New data
Solubility of HC Blue 18 for conducting toxicological studies was determined individually as part of the respective study.
Generally, the phenolic form of HC Blue 18 is practically insoluble in pure water and shows a medium to good solubility in standard organic solvents like MeOH, EtOH, isoPrOH, DMSO, DMF. The more polar anionic of HC Blue 18 shows good solubility in water above pH 9.

SCCS comment
The study report of water solubility determination was not submitted. It is not known whether the water solubility was determined by the EU Method A.6.

3.1.7 Partition coefficient (Log P_{ow})

Taken from SCCS/1560/15

Log P_{ow}: 2.33 +/- 1.30 (neutral); - 0.82 +/- 1.0 (mono-anionic form)

SCCS comment from the previous Opinion
It is not clear whether Log Pow was calculated or whether it was experimentally determined by EU Method A.8.

New data
Log P_{ow}: 3.72 (phenolic form) and Log P_{ow}: 0.66 (anionic form); calculated by "Molinspiration property engine v2018.10".

According to the Applicant, the anionic form has a lower calculated log P_{ow} value than the phenolic form. This translates into better water solubility for the anionic form, while the less polar phenolic form shows better solubility in the more lipophilic octanol.

3.1.8 Additional physical and chemical specifications

New data

Melting point:	The amorphous powder had no sharp melting point. It melted slowly in a range between 273 to 279 °C
Boiling point:	/
Flash point:	/
Vapour pressure:	/
Density:	/
Viscosity:	/
pKa:	6.31 (in 2.5% iso-PrOH, spectrophotometric determination)
Refractive index:	/
pH:	6.38 to 6.44 in deionized water

UV- Vis spectra

Taken from SCCS/1560/15

UV_Vis spectrum (ca.300nm-800 nm): λ_{max} ca. 410 nm and 625 nm

New data
According to the Applicant, HC Blue 18 exists in a protonated (phenolic) yellow coloured and deprotonated (anionic) violet-blue coloured form (Figure xxx, left). The protonation and deprotonation processes are reversible and do not influence the chemical stability of the dye.
The UV-Vis spectra of HC Blue 18 is dependent on the level of deprotonation and type of solvent (Figure 7, right). Additionally, the concentration of the dye in the solvent can also influence the spectrum as, for higher concentrations, this may lead to interactions between the dye molecules.

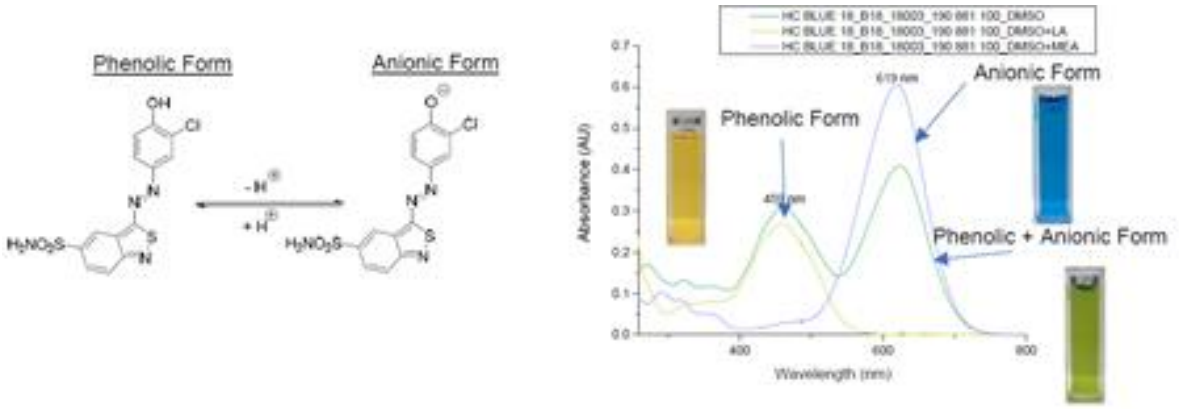


Figure 7. Anionic and phenolic form of HC Blue 18 (left). In pure DMSO (orange spectrum), both the anionic and phenolic form are present in the UV-Vis spectra (right)

The UV-Vis spectrum of HC Blue 18 in DMSO shows a double peak due to the presence of protonated and deprotonated dye in the solvent. In the other solvents used for UV-Vis measurement, the deprotonated anionic form is dominant prior to pH adjustment.

The absorbance maxima for HC Blue 18 in selected solvents are summarised in Table 6.

Table 6. Summary of solvents used for UV-Vis measurements of HC Blue 18. A shift in absorption maxima is visible depending on the solvent used

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)	450	573
	5% (v/v) iso-PrOH in water	450	565
	HPLC 50%(v/v) n-PrOH, 32% (v/v) AcCN, 8% (v/v) MeOH, 10% (v/v) H_2O	451	588
	DMSO	459	619

3.1.9 Homogeneity and Stability

Taken from SCCS/1560/15
0.5% FPK-145 (dissolved in monoethanolamine buffer containing isopropanol) was shown to be stable when mixed with 6% hydrogen peroxide (1:1).

New data
Alkaline Peroxide Stability
Alkaline peroxide stability of HC Blue 18 was determined using HPLC and UV-Vis, both using MEA buffer system at pH 10 and a 6% peroxide solution. The stability was determined using the batch HC Blue 18_18003 190 881 100 for both tests.

Stability of HC Blue 18 in alkaline peroxide with HPLC
Alkaline peroxide stability of HC Blue 18 over 45 min at room temperature (22°C) was determined in a buffered alkaline solution (MEA buffer at pH 10).

Method description: About 0.023 g of HC Blue 18 was dissolved in 100 mL buffer (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% H_2O_2 solution in water (ratio = 1:1 w/w) and a 44 min timer was started immediately. The mixture was stirred intensely for 20 sec and the t=0 was measured. 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 575 nm.
The stability of HC Blue 18 in presence of alkaline peroxide over 45 minutes, determined via HPLC, was found to be excellent with 98.84% for 254 nm and 99.63% for 575 nm. No new peaks were found in the HPLC data after 45 minutes for 254 and 575 nm under these conditions. According to the HPLC data, HC Blue 18 can be considered stable under alkaline peroxide conditions.

Stability of HC Blue 18 in alkaline peroxide with UV-Vis
Alkaline peroxide stability of HC Blue 18 over 45 min at room temperature (22°C) was determined in a buffered alkaline solution (MEA buffer at pH 10).
Method description: About 0.02 g of HC Blue 18 was dissolved in 400 mL buffer (4.5% MEA, 5.0% iso-PrOH in water; pH adjusted to 10 with HCl). 8 g of the freshly prepared dye solution was mixed with 8 g of a 6% H2O2 solution in water (ratio = 1:1 w/w) and a 44 min timer was started immediately. The mixture was stirred intensely for 20 sec and the t=0 was measured.
The sample was analysed directly after mixing with 6% peroxide solution (t=0), after 15, after 30 and after 45 minutes at room temperature (t=45 min). As reference, the dye solution was mixed with water instead of 6% peroxide solution and analysed as described above. The maximum of the dye peak for this solvent system was found to be 528 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 528 nm for t=0 (=100%). The test was repeated 3 times for batch HC Blue 18_18003 190 881 100.
The stability of HC Blue 18 in presence of alkaline peroxide over 45 minutes, determined via UV-Vis spectroscopy, was found to be excellent with a stability greater than 99.6%. The UV-Vis spectra does not show any changes within 45 min. According to the UV-Vis data, HC Blue 18 can be considered stable under alkaline peroxide conditions.

SCCS comment
The test substance, as indicated by the HPLC-PDA data submitted by the Applicant, is stable in the presence of alkaline peroxide for up to 45 minutes.

3.2 Exposure Assessment

3.2.1 Function and uses

HC Blue 18 is intended to be used as a direct hair colouring agent up to on-head concentration of 0.35% in non-oxidative as well as oxidative hair dye formulations.

3.2.2 Calculation of SED / LED

In its previous Opinion (SCCS/1560/15) the SCCS has calculated SED for oxidative and non-oxidation conditions as follows:

Oxidative conditions:
(0.7% formulation, on head concentration 0.35%)

Absorption through the skin	A	=	0.136 µg/cm²

Skin Area surface	SAS	=	580 cm^2
Dermal absorption per treatment	SAS x A x 0.001	=	0.079 mg
Average human body weight	=	60 kg	
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.0013 mg/kg bw**

Non-oxidative conditions:
0.35% formulation, on head concentration 0.35%

Absorption through the skin	A	=	0.791 µg/cm^2
Skin Area surface	SAS	=	580 cm^2
Dermal absorption per treatment	SAS x A x 0.001	=	0.459 mg
Typical body weight of human		=	60 kg
Systemic exposure dose (SED)	**SAS x A x 0.001/60**	**=**	**0.0076 mg/kg bw**

SCCS comment
The toxicological evaluation of HC Blue 18 can be found in the SCCS/1560/15 Opinion; in this Opinion the NOAEL is established at 25 mg/kg bw/d.

3.3 Safety evaluation (including calculation of the MoS)

3.3.1 Calculation of the Margin of Safety

MOS calculations under oxidative conditions
(0.7% formulation, on head concentration 0.35%)

Systemic exposure dose	SED	=	0.0013 mg/kg bw
No observed adverse effect level (90-day, oral, rat)	NOAEL	=	25 mg/kg bw/d
Bioavailability 65%*		=	16 mg/kg bw/d
Margin of Safety	**adjusted NOAEL/SED**	**=**	**12300**

* based on the toxicokinetic study (ref. 13)

MOS calculations under non-oxidative conditions
(0.35% formulation, on head concentration 0.35%)

Systemic exposure dose (SED)	SED	=	0.0076 mg/kg bw
No observed adverse effect level (90-day, oral, rat)	NOAEL	=	25 mg/kg bw/d
Bioavailability 65%*		=	16 mg/kg bw/d
Margin of Safety	**adjusted NOAEL/SED**	**=**	**2100**

* based on the toxicokinetic study (ref. 13)

3.3.2 Discussion

- Based on the additional physicochemical data provided by the Applicant, the SCCS agrees that the purity of the new commercial batches has been improved and only one impurity is detected at 575 nm (Impurity 3B). Impurity 3 B was chemically characterised by the Applicant and matches the structure of (E)-3-((3,5-dichloro-4-hydroxyphenyl)diazenyl)-benzo[c]isothiazole-5-sulfonamide.
- The SCCS agrees that both HC Blue 18 and the impurity 3B belong to Cramer Class III. Read-across results – and also QSAR results – indicate that the close analogues are negative for genotoxicity. Hence, it can be considered that impurity 3B is not of genotoxicological concern.
- The test substance, HC Blue 18, as indicated by the provided HPLC-PDA data, is stable in the presence of alkaline peroxide for up to 45 minutes.

4. CONCLUSION

1. In light of the data provided, does the SCCS consider HC Blue 18, safe when used in non-oxidative as well as in oxidative hair dye formulations up to a maximum on-head concentration of 0.35 %?

In light of the new physicochemical data provided, SCCS considers that the use of HC Blue 18 as an ingredient in non-oxidative as well as in oxidative hair dye formulations up to a maximum on-head concentration of 0.35% is safe.

2. Does the SCCS have any further scientific concerns with regard to the use of HC Blue 18 in cosmetic products?

SCCS considers HC Blue 18 as a moderate sensitiser.

5. MINORITY OPINION

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6. REFERENCES

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New References

SUBMISSION II, (ADDENDUM TO SUBMISSION I), 10 October 2022.

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.